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EFECTOS DE LA DIETA DE CAFETERÍA Y DEL CONSUMO VOLUNTARIO DE ALCOHOL EN LA EXPRESIÓN DE GENES RELACIONADOS CON PLASTICIDAD NEURONAL EN EL SISTEMA DE RECOMPENSA

Tesis sometida a la consideración de la Comisión del Programa de Estudios de Posgrado en Biología para optar al grado y título de Maestría Académica en Biología con énfasis en Genética y Biología Molecular

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DEDICATORIA

Este trabajo va dedicado a la perseverancia, la fortaleza y la resiliencia de las mujeres que han decidido permanecer fuertes y valientes en el mundo de la ciencia, a pesar de haber sido juzgadas sin razón alguna.

De una forma muy especial, lo dedico a mi esfuerzo, a mi resiliencia, a mis deseos de superación, y a mi pasión por la ciencia, esas cualidades son las que permitieron que hoy haya llegado donde estoy.

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RESUMEN

El aumento de la obesidad a nivel global y regional representa un problema de salud pública, dadas las complicaciones crónicas asociadas a esta condición. La obesidad es un rasgo complejo en el que intervienen factores genéticos y ambientales. Entre los factores de riesgo asociados a obesidad se encuentra el cambio en los patrones alimentarios de la sociedad occidental, caracterizado principalmente por el aumento en el consumo y disponibilidad de alimentos con un alto contenido energético, grasa y azúcar. Además, se ha sugerido que el incremento en el consumo de alcohol contribuye con el aumento en las cifras de obesidad, por lo que este factor debería ser tomado en cuenta en estudios de obesidad tanto a nivel preclínico como epidemiológico. En esta tesis implementamos un modelo preclínico de obesidad para estudiar los efectos independientes y la interacción entre una dieta altamente palatable y energéticamente densa típica de las sociedades occidentales conocida como dieta de cafetería (CAF) y el consumo voluntario de etanol (ALC) sobre los parámetros biométricos, nutricionales, conductuales y neuroquímicos (expresión de genes y proteínas) implicados en la plasticidad neuronal en varias regiones cerebrales del sistema de recompensa. Siguiendo un diseño factorial de 2x2, se expuso a 50 ratas Wistar macho durante 9 semanas a la combinación de los siguientes factores: Dieta (dieta estándar vs. CAF) y Etanol (agua vs. ALC). La ingesta de alimentos y el peso corporal se midieron diariamente, mientras que los efectos conductuales se evaluaron en la prueba de campo abierto (OFT) al finalizar la exposición a los tratamientos. Finalmente, se eutanasiaron los animales, se disecaron los cerebros y se analizaron los genes y proteínas en regiones del sistema de recompensa como la corteza prefrontal medial (CPFm), el núcleo accumbens (NAc), el estriado dorsal (ED) y el hipocampo (HPC). Los resultados mostraron que la CAF causó hiperfagia, aumentó el peso corporal y la acumulación de grasa perigonadal y abdominal, mientras que el ALC solo incrementó la grasa de la zona abdominal. A nivel conductual, la CAF, y en menor medida el ALC, indujeron un efecto de tipo ansiolítico sin afectar la actividad psicomotora general. A nivel cerebral, la CAF y el ALC tuvieron efectos independientes y de interacción sobre la expresión génica y los niveles de proteínas. La CAF indujo un incremento de los niveles de ARNm de CREB en el HPC y una disminución en la CPFm. El ALC, por el contrario, reguló negativamente la expresión de CREB, CRFR1 y TrkB en el HPC. En cuanto al contenido de proteínas, ambos tratamientos redujeron en la misma proporción la proteína CREB en el ED. Sin embargo, el ALC, y en menor medida la CAF, aumentaron la relación pCREB/CREB en la CPFm y el ED. Considerando las diferencias observadas en los niveles de ARNm y proteína, el parámetro más sensible a ambos tratamientos fue CREB, y en segundo lugar, la expresión de CRFR1. Nuestros hallazgos sugieren que nuestro modelo es útil y robusto para estudiar los efectos neuroconductuales de una dieta occidentalizada moderna en el desarrollo de trastornos metabólicos y obesidad.

ABSTRACT

The increase in global and regional obesity represents a public health problem, given the chronic complications associated with this condition. Obesity is a complex trait influenced by genetic and environmental factors. The change in dietary patterns in Western society is among the risk factors associated with obesity, which are characterized mainly by an increase in the consumption and availability of high-energy, fatty, and sugary foods. Additionally, it has been suggested that increased alcohol consumption contributes to the rising rates of obesity, so this factor should be considered in both preclinical and epidemiological obesity studies. In the present thesis, we implemented a preclinical model of obesity to study the independent and interactive effects of a highly palatable and energetically dense diet typical of Western societies –known as cafeteria diet (CAF)– and voluntary ethanol consumption (ALC), on obesity-related biometric, nutritional, behavioral, and neurochemical parameters. Fifty male Wistar rats were exposed for 9 weeks to the combination of Diet (standard vs. CAF) and Alcohol (water vs. ALC) factors. Food intake and body weight were scored daily, whereas the behavioral effects were assessed after the nine-weeks period. Gene expression and protein levels were analyzed in brain regions of the reward system such as the medial prefrontal cortex (mPFC), the nucleus accumbens (NAc), the dorsal striatum (DS), and the hippocampus (HPC). We found that CAF caused hyperphagia, body weight gain, and body fat accumulation, while both treatments increased abdominal fat. In the open-field test, CAF, and to a lesser extent ALC, induced an anxiolytic-like effect. At the brain level, CAF changed CREB expression in opposite directions between the mPFC and the HPC, decreasing and increasing it, respectively. ALC, in contrast, downregulated CREB, CRFR1, and TrkB expression in the HPC. Both treatments equally reduced CREB protein in the DS. ALC, and to a lesser extent CAF, increased the pCREB/CREB ratio in the mPFC and the DS. Altogether, CREB and CRFR1 were the most sensitive genes to the treatments. Our preclinical model proved to be useful for studying the neurobehavioral effects of a modern Westernized diet and alcohol consumption on the risk of suffering metabolic disorders and obesity.

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INTRODUCCIÓN

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LISTA DE ABREVIATURAS

ACTH: hormona adenocorticotrópica

ALC: alcohol

BDNF: factor neurotrófico derivado del cerebro

CAF-ALC: cafetería-alcohol

CAF: cafetería

CPFm: corteza prefrontal medial

CREB: elemento de respuesta al cAMP

CRF: factor liberador de corticotropina

CRFR1: receptor tipo 1 del factor liberador de corticotropina

CT: control

DPN: día post-natal

ED: estriado dorsal

HPA: sistema adrenal-pituitario-hipotalámico

HPC: hipocampo

HPRT: hipoxantina fosforribosiltransferasa 1

NAc: núcleo accumbens

OH: alcohol

pCREB: CREB fosforilada (forma activa)

RRF: campo retro-rubral

SNc: sustancia negra

TrkB: receptor tirosín-quinasa relacionado con tropomiosina B

VTA: área tegmental ventral

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INTRODUCCIÓN

Obesidad y su relación con la ingesta de alimentos de alta palatabilidad y de alcohol:

Los cambios en los estilos de vida de las personas durante los últimos 40 años han llevado a un cambio en la alimentación, que se caracteriza por un aumento en el consumo de alimentos altos en grasa y carbohidratos simples y que poseen una alta palatabilidad, pero también una alta densidad energética. Lo anterior, contribuye con el dramático aumento en los índices de obesidad, tanto en países desarrollados como en vías de desarrollo (Morris *et al.*, 2015). Algunas estimaciones indican que aproximadamente mil millones de personas alrededor del mundo tienen sobrepeso, y 400 millones de estas presentan obesidad (Morris *et al.,* 2015). En nuestro país la obesidad ha aumentado considerablemente en los últimos años. Según datos de población urbana costarricense, aproximadamente el 63% de las personas entre 15-65 años muestran alteraciones del peso corporal (Fisberg *et al.,* 2015; Kovalskys *et al*., 2018). A nivel nacional pero en población escolar, se reportó una prevalencia de 34% de sobrepeso y obesidad (Ministerio de Salud y Ministerio de Educación Pública, 2017).

La palatabilidad es una definición hipotética necesaria para determinar los aspectos hedónicos del sabor, olor, sabor, textura, etc. de los alimentos (Rogers, 1990), y está estrechamente relacionada con la densidad de energía de los mismos. De hecho, los alimentos con alto contenido energético son, con frecuencia, percibidos como "sabrosos", y viceversa (Drewnowski, 1998). La palatabilidad también se asocia con un consumo excesivo de alimentos, trayendo consigo consecuencias para la salud humana, tales como la obesidad (Bellisle *et al.,* 1984; de Castro *et al.,* 2000; Yeomans *et al.,* 2004).

La obesidad es un trastorno metabólico que se caracteriza por una acumulación excesiva de tejido adiposo. Su desarrollo involucra una interacción compleja entre la susceptibilidad genética individual y el ambiente en el que se desenvuelven los individuos. Por esto se dice que el consumo de una dieta alta en alimentos energéticamente densos, ricos en grasa y azúcar, unido a estilos de vida sedentarios y con altos niveles de estrés

que acompañan la vida moderna occidental promueven el aumento en las cifras de obesidad anteriormente mencionadas (Lyon & Hirschhorn, 2005; Morris *et al.,* 2015; Popkin & Reardon, 2018).

Otro de los factores de riesgo asociados a la obesidad en estudios epidemiológicos y cuya contribución ha sido poco estudiada, es el consumo de alcohol, es decir, no existen suficientes estudios que incluyan al alcohol como parte de la dieta en estudios epidemiológicos de obesidad. El alza en las cifras de obesidad en el mundo con el paso de los años, coincide con los incrementos en el consumo de alcohol, por lo que se ha sugerido que el consumo de bebidas alcohólicas podría contribuir al riesgo de obesidad y debería ser tomado en consideración tanto en estudios poblacionales como preclínicos (Yeomans, 2010; World Health Organization, 2014).

En Costa Rica la prevalencia de consumidores activos de alcohol es de 27.9% (www.datosabiertos.iafa.org), siendo la prevalencia más alta en el grupo etario de 20-39 años. El aporte calórico del alcohol tiene un valor nutricional bajo y es aditivo a la ingesta total diaria, pues su consumo no reduce la ingesta de los otros macronutrientes (Sutter *et al*., 1997). Asimismo, el alcohol puede aumentar el consumo de alimentos, ya sea porque magnifica la percepción de apetito en presencia de comida o por su efecto psicoactivo que puede llevar a modificar la conducta de ingesta (Yeomans *et al.,* 1999).

El consumo excesivo de alimentos de alta palatabilidad, o sabrosos, combinados con la ingesta de alcohol pueden resultar en alteraciones de los parámetros biométricos relacionados con el peso corporal, como lo son un aumento en la grasa corporal (Blumenthal & Gold, 2010). El consumo de los alimentos mencionados, unido al consumo de alcohol, pueden inducir cambios neuroplásticos y llevar al individuo a la sobreingesta de estos, debido a una activación del sistema mesocorticolímbico-dopaminérgico (Berthoud, Lenard & Shin, 2001; Morris *et al.* 2015).

El sistema de recompensa como regulador de la conducta de ingesta:

La recompensa es cualquier actividad, sentimiento, sensación, etc., que nos motiva a repetir una acción, e involucra conductas como por ejemplo la búsqueda de alimento o de sustancias psicoactivas (Schultz, 2010). El sistema mesocorticolímbico-dopaminérgico, también llamado sistema de recompensa (Shizgal, 1997) es un sistema neural que procesa recompensas, en sus diferentes modalidades y, por lo tanto, funciona como una escala común a través de la cual se pueden contrastar diversos estímulos que resultan gratificantes. Dicho sistema está conformado por un conjunto de neuronas dopaminérgicas, casi todas ubicadas en la parte ventral del mesencéfalo. Las neuronas dopaminérgicas mesodiencefálicas forman la sustancia negra (SNc), el área tegmental ventral (VTA) y el campo retro-rubral (RRF). Además, se incluye el sistema nigroestriatal, que se origina en el SNc y extiende sus fibras hacia el núcleo caudado-putamen (Smith & Villalba, 2008). El sistema de recompensa incluye la vía mesolímbica y mesocortical, que surgen del VTA y se ha sugerido que modulan el comportamiento relacionado con las emociones, el refuerzo, la conducta motivada y el aprendizaje asociativo (D´Ardenne *et al.,* 2008; Phillips, Vacca & Ahn, 2008). El sistema mesolímbico dopaminérgico incluye las neuronas dopaminérgicas del VTA que proyectan principalmente al núcleo accumbens (NAc), así como a la amígdala y el hipocampo. Por otro lado, el sistema dopaminérgico mesocortical que incluye aquellas que extienden sus fibras en el córtex prefrontal, cingulado y perirrinal desde el VTA. Debido a la superposición entre estos dos sistemas, a menudo se les conoce colectivamente como el sistema mesocorticolímbico (Wise, 2005) (**Fig. 1**).

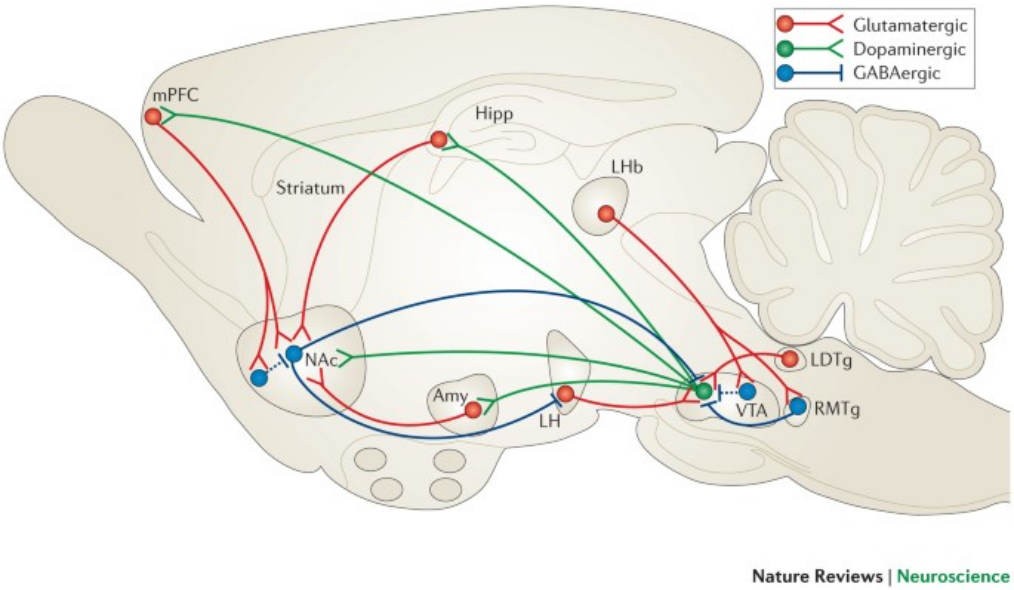

Figura 1. Estructuras que conforman el sistema de recompensa en el cerebro de rata. Tomado de Russo y Nestler, 2013.

La mayor parte de la motivación, incluso la búsqueda de comida o agua, se aprende (Changizi, McGehee & Hall, 2002). La motivación es regresar a las recompensas experimentadas y a las señales contextuales que marcan el camino hacia tales recompensas. Es principalmente a través del sistema mencionado que se forman asociaciones de estímulo-recompensa, y estas pueden permanecer potentes durante algún tiempo, incluso después de que la recompensa se haya devaluado por la ausencia de estados de impulso apropiados, como hambre o sed (Changizi, McGehee & Hall, 2002).

Existe un ciclo recientemente descrito en dependencias llamado ciclo o circuito de la dependencia (**Fig. 2**). La dependencia representa una desregulación de los circuitos motivacionales causada por una repetición de hábitos, déficit de recompensas y exceso de estrés. Los efectos gratificantes de las sustancias motivadoras actúan igual que las sustancias de abuso, ya que estas últimas secuestran el sistema que procesa las recompensas naturales. En este ciclo existe una etapa de atracón/intoxicación, la cual implica cambios en la neurotransmisión dopaminérgica y de péptidos opioides en los ganglios basales del cerebro. También hay una etapa posterior de retiro/estado afectivo

negativo, donde aumentan los estados emocionales negativos y las respuestas disfóricas y similares al estrés, relacionadas con la abstinencia, la tolerancia y un escalamiento en el consumo Finalmente la etapa de anticipación/preocupación implica la desregulación de las proyecciones de la corteza prefrontal y la ínsula, que incluyen glutamato, a los ganglios basales y a la amígdala extendida (Koob & Volkow, 2016) y se relaciona con un sesgo atencional hacia las señales contextuales asociadas a la recompensa y con la pérdida del control inhibitorio, promoviendo conductas compulsivas.

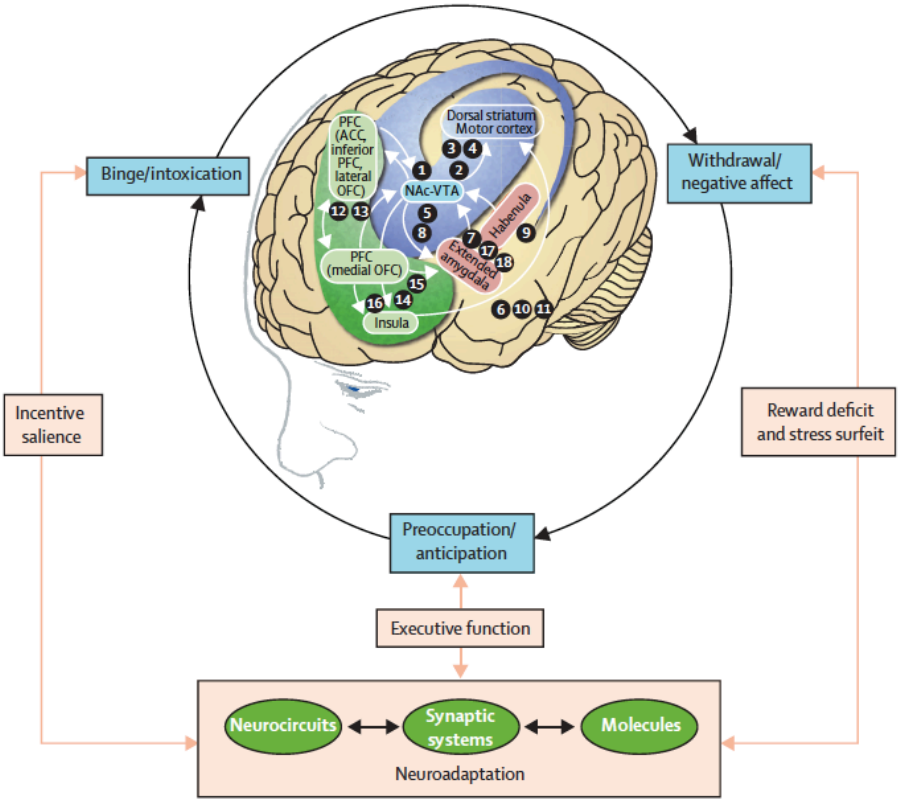

Figura 2. Circuito de la dependencia y las respectivas regiones cerebrales involucradas. Tomado de Koob & Volkow, 2016.

Durante el consumo prolongado de alimentos de alta palatabilidad, o durante el consumo de alcohol, se activan vías neuronales que involucran distintos sustratos cerebrales relacionados con el sistema de recompensa antes mencionado. Algunos de estos sustratos cerebrales son la corteza prefrontal medial (CPFm), el estriado dorsal (ED) y el NAc

(German & Manaye, 1993; Barrett, Mercer & Morgan, 2016). Dicha activación podría estar influenciada por alteraciones en la expresión de genes (Olsen, 2011). Se ha reportado que el consumo de alimentos de alta palatabilidad y la ingesta de sustancias psicoactivas como el alcohol, pueden inducir cambios en la expresión de genes asociados con plasticidad neuronal. Algunos de los genes involucrados son: el factor neurotrófico derivado del cerebro (BDNF) y su receptor, el receptor de la tropomiosina kinasa B (TrkB), la proteína de unión al elemento de respuesta al AMPc (CREB), el factor liberador de corticotropina (CRF) y su receptor tipo 1 (CRFR1).

La proteína BDNF se expresa altamente en el cerebro, incluidas las regiones hipocampales y corticales, y tiene funciones fundamentales en el mantenimiento de las neuronas en el sistema nervioso central (SNC). BDNF es una neurotrofina involucrada en la supervivencia y diferenciación de distintas poblaciones celulares, y actúa sobre ciertas neuronas del SNC y periférico, ayudando a mantener la supervivencia de las neuronas existentes (Huang & Reinchardt, 2001; Chao, 2003; Lu *et al*., 2013; Ohira & Hayashi, 2009). Recientemente, describimos que los niveles de ARNm de BDNF en el NAc correlacionan negativamente con parámetros relacionados con el peso corporal y el consumo de alimentos en ratas expuestas a una dieta de alta palatabilidad (Vindas-Smith *et al.*, 2022). Previamente, se ha descrito que en animales que no expresan BDNF en el VTA muestran sobreingesta de alimento cuando se exponen a una dieta alta en grasa (Cordeira *et al.*, 2010)

El factor de transcripción CREB es un factor que regula la expresión de genes con importancia funcional en las neuronas dopaminérgicas (Wang *et al.,* 2018). Estudios en modelos animales han demostrado que la exposición al alcohol en ratas, causa un aumento en la fosforilación de CREB (forma activa) en la amígdala, lo que aumenta a su vez los niveles de BDNF, disminuyendo la ansiedad (Pandey *et al.,* 2008). Lo contrario se observa cuando las ratas consumen alcohol de forma crónica (Kyzar & Pandey, 2015). Igualmente se ha observado que dietas altas en grasa disminuyen la fosforilación de CREB en el NAc (Bocarsly & Avena, 2013).

Por otro lado, CRF y su receptor CRFR1 (Speliotes *et al*., 2010) actúan como mediadores de respuestas endocrinas, autónomas, conductuales e inmunes al estrés (Tache *et al.,* 2004). La proteína CRF es un péptido liberado por el hipotálamo ventromedial al sistema porta-hipofisiario, a través de vasos sanguíneos que llegan a la adenohipófisis donde promueve la secreción de la hormona adenocorticotrópica (ACTH), ayudando al cuerpo a reaccionar en situaciones de estrés (Roca, 2010). El péptido CRF participa en la activación del sistema adrenal-pituitario-hipotalámico (HPA), que lleva a la liberación de ACTH con el fin de estimular la síntesis y secreción de glucocorticoides desde la corteza adrenal (Adam & Epel, 2007; Morris *et al.,* 2014). Estos glucocorticoides tienen un efecto en la regulación de la ingesta de alimentos, pues tienen efectos anorexigénicos y termogénicos, como mecanismo de respuesta al estrés, al aumentar el gasto energético por una activación del sistema nervioso simpático (Morris *et al.,* 2014). Algunos glucocorticoides ejercen efectos diabetogénicos al interferir con la acción de la insulina, pues inhiben la secreción de esta desde las células β del páncreas (Adam & Epel, 2007). Otros glucocorticoides como el cortisol tienen efectos sobre el sistema de recompensa, en respuesta al aumento de los niveles de insulina, leptina y neuropéptido Y (Morris *et al.*, 2014). En el cerebro, un aumento de cortisol tras el aumento en niveles de insulina podría llevar a una sobreestimulación del sistema de recompensa, trayendo consigo sentimientos de placer asociados a ciertos alimentos (Adam & Epel, 2007).

Todos estos genes mencionados anteriormente participan en la modulación de vías neuronales asociadas con procesos de plasticidad neuronal. Estudios han demostrado que algunos factores neurotróficos, tales como el BDNF, median cambios permanentes en circuitos neuronales relacionados con la memoria y el aprendizaje, y a su vez, estos cambios plásticos juegan un papel relevante en la dependencia de sustancias y en la búsqueda de alimentos de alta palatabilidad (Wise, 2000). El consumo de alcohol puede llevar a un trastorno de dependencia en algunos individuos vulnerables, que en situaciones de abstinencia promueve la activación del eje HPA y aumenta la liberación de CRF, generando un desbalance en las vías que regulan la respuesta al estrés, alteraciones similares se han observado con el consumo excesivo de alimentos palatables (Fernández-Espejo, 2002). Sin embargo, al estudiar la actividad de los genes es importante tomar en

cuenta que los niveles de ARNm no necesariamente correlacionan con los niveles de proteína. Además, los efectos moleculares varían según la región del cerebro analizada por lo que es posible obtener diferencias en expresión génica y en proteínas al analizar diferentes regiones del sistema de recompensa (Morris *et al*., 2015).

La dieta de cafetería y el consumo de alcohol para el estudio de efectos biométricos y neuroconductuales en modelos animales:

Los humanos y los roedores tienen regiones cerebrales similares involucradas en el control del balance energético, por lo que los roedores se pueden emplear para responder interrogantes que en humanos serían casi imposibles de resolver (Campos-Rosini, Sánchez-Ramos & de Moraes, 2012). Para generar modelos animales de obesidad se utilizan diferentes metodologías. El modelo de la dieta de cafetería fue desarrollado por Sclafani & Springer (1976), sin embargo, su uso se ha extendido en las últimas dos décadas y ha sido validado como un modelo que refleja los patrones alimentarios de las sociedades occidentales, y que genera alteraciones similares al síndrome metabólico observado en humanos (Sampey *et al*., 2011; Gutiérrez-Martos *et al*., 2018). Dicha dieta consiste en la administración de alimentos de consumo humano altamente procesados, ricos en grasa y azúcar, que se consiguen en un supermercado o cafetería. Esta dieta puede ser modificada para estudiar el impacto de otros estímulos recompensantes, como el acceso voluntario a sustancias psicoactivas como el alcohol.

Dada la importancia de la obesidad en el desarrollo de otras patologías crónicas, como la diabetes tipo 2, que tienen efectos sobre la calidad de vida de las personas y generan altos costos a la seguridad social, surge la necesidad de ahondar en los mecanismos neurofisiopatológicos que contribuyen al desarrollo de esta condición. Es por esto que este proyecto pretendió responder a la pregunta sobre cuál es el efecto de una dieta de alta palatabilidad y del consumo voluntario de alcohol, sobre los parámetros biométricos y conductuales asociados con la obesidad, así como estudiar cambios en la expresión de genes involucrados en plasticidad neuronal en el sistema de recompensa. La exposición a la dieta de alta palatabilidad y al consumo de alcohol podría inducir cambios en la

expresión de los genes ya mencionados, y estas alteraciones podrían asociarse con un fenotipo de obesidad/dependencia de sustancias que eventualmente llevarían a un consumo excesivo de alimentos de alta palatabilidad y/o alcohol.

OBJETIVOS

Objetivo general:

Evaluar los efectos de una dieta de alta palatabilidad y del consumo voluntario de alcohol en la expresión de genes involucrados en procesos de plasticidad neuronal, para el desarrollo de un modelo preclínico de obesidad/dependencia de sustancias.

Objetivos específicos:

1. Evaluar el efecto de la dieta de cafetería y del consumo voluntario de alcohol sobre parámetros biométricos asociados con obesidad.

2. Caracterizar el efecto de la dieta de cafetería y del consumo voluntario de alcohol sobre la respuesta psicomotora de los animales.

3. Determinar alteraciones en el sistema mesocorticolímbico-dopaminérgico mediante cambios en la expresión de BDNF y su receptor TrkB, CREB, CRF y su receptor CRF1, en el estriado dorsal, el núcleo accumbens, la corteza prefrontal medial y el hipocampo, inducidos por la dieta de cafetería y el consumo de alcohol.

4. Identificar asociaciones entre los niveles de ARNm y de proteína de BDNF, CREB y pCREB en el estriado dorsal, el núcleo accumbens y la corteza prefrontal medial, y los parámetros biométricos y conductuales.

Effects of the cafeteria diet and voluntary alcohol consumption on the expression of genes related to neuronal plasticity in the reward system

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Abstract

Here, we implemented a preclinical model of obesity to study the independent and interactive effects of a highly palatable and energetically dense diet typical of Western societies –known as cafeteria diet (CAF)– and ethanol consumption (ALC), on obesity-related biometric, nutritional, behavioral, and neurochemical parameters. Fifty male Wistar rats were exposed for 9 weeks to the combination of Diet (standard vs. CAF) and Alcohol (water vs. ALC) factors. Food intake and body weight were scored daily, whereas the behavioral effects were assessed after the nine-week period. Gene expression and protein levels were analyzed in brain regions of the reward system such as the medial prefrontal cortex (mPFC), the nucleus accumbens (NAc), the dorsal striatum (DS), and the hippocampus (HPC). We found that CAF caused hyperphagia, body weight gain, and body fat accumulation, while both treatments increased abdominal fat. In the open-field test, CAF, and to a lesser extent ALC, induced an anxiolytic-like effect. At the brain level, CAF changed CREB expression in opposite directions between the mPFC and the HPC. ALC, in contrast, downregulated CREB, CRFR1, and TrkB expression in the HPC. Both treatments equally reduced CREB protein in the DS. ALC, and to a lesser extent CAF, increased the pCREB/CREB ratio in the mPFC and the DS. Altogether, CREB and CRFR1 were the most sensitive genes to the treatments. Our preclinical model proved to be useful for studying the neurobehavioral effects of a modern Westernized diet and alcohol consumption on the risk of suffering metabolic disorders and obesity.

INTRODUCTION

Obesity is a metabolic disorder characterized by an excessive accumulation of adipose tissue that impairs human health (Blüher, 2020). It is caused by the complex interaction of genetic, metabolic, and environmental factors (Tremblay & Hamet, 2019). Environmental factors are particularly significant, considering that modifications in people's lifestyles over the last 40 years have led to changes in eating patterns, characterized by an increase in the consumption of highly palatable, energy dense foods high in fat and simple carbohydrates (Morris *et al.*, 2015).

The omnipresence and affordability of ultra-processed and hyperpalatable foods are hallmarks of our current obesogenic environment. Palatability refers to the hedonic value of food, conferred by the food's organoleptic properties (i.e., taste, smell, flavor, and texture), as well as the content of fat, sugar, and sodium, along with individual differences in preference and response (Johnson & Wardle, 2014). Energy-dense foods are often perceived as "tasty", and it is known that this kind of food is associated with excessive food consumption, which in turn has consequences for human health, leading to weight gain, obesity, and its associated metabolic comorbidities, such as diabetes, hypertension, and cardiovascular diseases, among others (de Castro *et al.*, 2000; Yeomans *et al.*, 2004).

Another risk factor associated with obesity but often neglected and little explored in both epidemiological and preclinical studies, is alcohol consumption. The rise in global obesity rates overlaps with the increases in alcohol consumption, which is why it has been suggested that the consumption of alcoholic beverages should be considered as a dietary risk factor in obesity studies (Yeomans, 2010; World Health Organization, 2014). As a dietary factor, alcohol contributes 7 kcal/g, having more energy content than proteins and carbohydrates (Gazdzinski & Durazzo, 2013). However, given that the caloric intake of alcohol has little nutritional value and is additive to total daily energy intake, its consumption does not reduce the intake of other macronutrients (Sutter *et al.*, 1997). Likewise, alcohol can increase food consumption, either by magnifying the perception of appetite in the presence of food or through its psychoactive effects, which can lead to changes in eating behavior (Yeomans *et al.*, 1999), promoting weight gain due to its low satiating capacity. Furthermore, alcohol could be a risk factor for body fat accumulation (Blumenthal & Gold, 2010), as alcohol consumption, when combined with palatable food, is associated with overeating energy in the form of fat (Tremblay & St-Pierre, 1996), and alcohol metabolism inhibits fat oxidation (Sonko *et al*., 1994). Moreover, peripheral and central signals involved in the regulation of appetite and energy homeostasis, also influence the physiological effects of alcohol drinking (Brutman *et al*., 2020).

Both palatable food and alcohol are well-known reinforcers, which means that they can activate the mesocorticolimbic-dopaminergic system, also called the reward system

(Berthoud, Lenard y Shin, 2001; Morris *et al.,* 2015). The reward system is a neural system that serves as the central regulator of motivation, learning based on rewards, and decision-making (Shizgal, 1997). This system includes, the nucleus accumbens (NAc), dorsal striatum (DS), hippocampus (HPC), and medial prefrontal cortex (mPFC), among other brain regions. Dopamine (DA) acts as the primary neurotransmitter associated with rewards in these regions (Koob & Volkow, 2010). Persistent consumption of high-fat and high-sugar palatable foods, as well as alcohol drinking, leads to enduring changes in the brain's reward system, a phenomenon known as long-term neuroplasticity that enables the brain to change in response to environmental stimuli. These neuroplastic adaptations could be induced by alterations in gene expression, serving as an initial stage mechanism in the establishment of maladaptive behaviors (Olsen, 2011).

The brain-derived neurotrophic factor (BDNF) is one of the most important signaling molecules involved in neuroplasticity, which binds to the tropomyosin receptor kinase B (TrkB) to mediate cellular growth, survival, and differentiation (Cordeira *et al*., 2011). Likewise, the corticotrophin-releasing factor (CRF) and its receptor type 1 (CRFR1) control the stress response and stress-related plasticity and modulate the consumption of highly palatable foods (Iemolo *et al*., 2013). Both pathways activate intracellular regulators, such as the cAMP response element-binding protein (CREB). CREB has been found regulating the expression of several genes involved in neuroplasticity (McClung & Nestler, 2008), including BDNF. Several studies have shown that these genes mediate permanent changes in neural circuits related to memory and learning. In turn, these plastic changes play a significant role in substance dependence and persistent food-seeking, especially highly palatable food (Wise, 2000). This study aimed to assess the independent and interactive effects of a highly palatable and energetically dense diet typical of Western societies –known as cafeteria diet– and voluntary ethanol consumption, on obesity-related biometric, nutritional, behavioral, and neurochemical parameters.

MATERIALS AND METHODS

Animals and housing conditions

Fifty male Wistar Hannover rats (HsdBrlHan: WIST) from the Laboratorio de Ensayos Biológicos at the Universidad de Costa Rica were transported to our animal facility room at postnatal day (PND) 22 after weaning. The animals were group-housed in polycarbonate cages according to their littermates of origin (2-4 per cage) and acclimatized for one week in a controlled room with a 12:12 light/dark cycle (lights on at 08:00), with free access to standard food (LabDiet 5010) and water. A handling protocol was carried out before experimental testing. The sample size (*n*) was calculated based on the magnitude of the effects obtained in our previous experiment using the cafeteria diet model (Vindas-Smith *et al.,* 2022), in which significant differences were found with *n* = 8 animals per group. For this study, we increased the sample size to 11-13 animals per group to enhance the statistical power. All experimental procedures were conducted in accordance with the guidelines of the Ministry of Science and Technology for the Care and Use of Laboratory Animals and received approval from the Institutional Committee for the Care and Use of Animals of the Universidad de Costa Rica (CICUA-034-2019).

Experimental design

The experimental design is depicted in **Fig. 1**. Briefly, rats were individually housed at PND 28, and divided into four groups counterbalanced by body weight, food and water intake, and the dam, in order to minimize potential early effects resulting from differences in maternal care and to maintain genetic variability among the four experimental groups, avoiding all pups from a single mother being placed in the same experimental group. The control group (STD-W, *n* = 11) received the standard diet (LabDiet 5010). The alcohol group (STD-OH, *n* = 13) also received the standard diet, and a 10% v/v alcohol solution. The cafeteria group (CAF-W, *n* = 13) had access to the standard diet, with a three-changing combination of food items usually consumed by people. The cafeteria-alcohol group (CAF-OH, *n* = 13) received the same diet as the CAF group with free access to the 10% v/v alcohol solution. All experimental groups had continuous access to water.

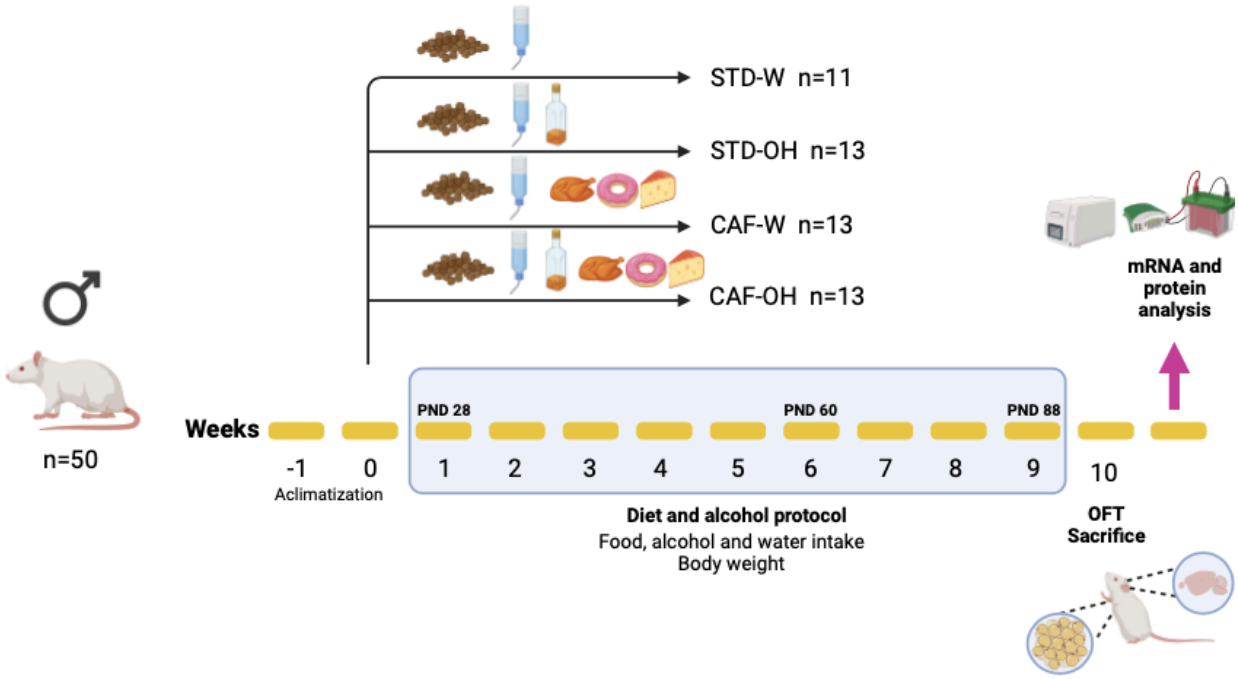

Figure 1. Study experimental design. After one acclimatization week, rats were single-housed to record food and water intake. Body weight, food and water intake, and the dam were used to balance the group's allocation randomly. Wistar rats were fed either a cafeteria or a standard diet for 9 weeks. All rats had free access to water, and the STD-OH and CAF-OH groups received 10% v/v ethanol during the feeding protocol. Body weight, food, and liquid intake (i.e., water and alcohol) were scored daily from Monday to Saturday. At the end of the 9th week, the open field test was performed for behavioral assessment. After euthanizing the animals, the abdominal and perigonadal fat deposits were dissected and weighed to determine body fat accumulation. Finally, brain tissues involved in reward-related processes were isolated to quantify the mRNA and protein expression levels. OFT, open field test. PND, post-natal days. Figure created with BioRender.com©.

All animals were exposed to their respective treatments for 9 weeks. Body weight, food consumption, and liquid (i.e., water and alcohol) intake were recorded six times a week from Monday to Saturday. At the end of the 9-week protocol, a behavioral analysis was conducted using the open field test (OFT) to assess spontaneous exploratory activity and anxiety-like behaviors. Finally, the animals were euthanized by decapitation, and both fat and brain tissues were collected. The entire experimental testing period covered the

transition from adolescent to young adulthood for the animals, spanning from PND 28 to PND 88 (Schneider, 2013)

Diets and food intake measurements

The standard chow diet provided 3.4 kcal/g and consisted of 58.2% of energy from carbohydrates, 28.7% from protein, and 13.1% from fat. The cafeteria diet was comprised of a combination of three palatable food items along with chow pellets. These combinations were prepared using a total of 18 food items, including cookies, sausages, wafers, cheese, cereals, among others, all of which had high fat, sugar and/or sodium content. The selection of these food items was based on our previous experiment (Vindas-Smith *et al*., 2022), a study addressing energy intake in Latin-American countries, including Costa Rica (Kovalskys *et al*., 2018), as well as other similar studies that utilized the cafeteria diet model (Pini *et al*., 2017; Sampey *et al*., 2011; Shafat *et al*., 2009). The three food combinations were changed daily (except Sundays) to ensure minimal repetition and to maintain variety of the cafeteria diet. All cafeteria-fed rats received the same amount of a given food for a particular combination. The energy derived from the cafeteria diet varied daily but in average provided 42% energy from carbohydrates, 45% from fats, and 13% from proteins. For groups receiving exclusively the standard food, an amount meeting the nutritional requirements of laboratory rats was provided (approximately 30-40 g/day), following the guidelines of the National Research Council Subcommittee on Laboratory Animals' Nutrition (1995). All food items were weighed before being presented to the animals, and any remaining food was weighed the following day. All leftovers were meticulously collected. The difference between the amount of food presented and the amount consumed was adjusted for food weight variations resulting from environmental conditions. For humidity correction, the same foods that were presented to the animals were weighed and placed in a polycarbonate cage with wood shavings, but without any animals. The corrected amount of each food consumed was used to calculate energy (kcal) and macronutrient intake according to the information provided by the manufacture's nutritional label.

Alcohol self-administration and liquid intake

Alcohol exposure was done following the two-bottle choice paradigm. For this, animals were exposed to a 10% v/v ethanol solution (**Fig. 1**) with voluntary access to both a water bottle and an alcohol bottle, both placed on the cage rack. Every day, the positions of the bottles were alternated to avoid conditioned place preference. Alcohol and water consumption were measured daily by recording the volume consumed during the exposure days. Alcohol and water intake were also corrected for evaporation or dripping, as described for food intake correction.

Biometric parameters

All rats were weighed daily on a scale from Monday to Saturday to obtain weekly measurements of body weight, as well as body weight gain, and final body weight. After euthanasia, perigonadal and abdominal fat depots were isolated and weighed to evaluate body fat accumulation after exposure to the treatments. Total white adipose tissue (WAT), expressed as a percentage of animal's body weight, was calculated as follows: [(perigonadal fat (g) + abdominal fat (g)) / final body weight (g)] x 100.

Open field test

Each animal was subjected to the OFT twice on consecutive days. The open field arena consisted of a square black wooden chamber (55 cm x 55 cm x 40 cm) used for the assessment of spontaneous locomotor activity and anxiety-related behaviors, as previously described (Rojas Carvajal *et al.,* 2019). Four rats were tested simultaneously in separate rooms dimly illuminated with two 25W red bulb located 150 cm above the arena (~10 lux). All rats were individually placed in the center zone of the arena and allowed to explore it for 15 minutes. All sessions were recorded on video, and we used the Any-Maze software v4.72 (Stoelting Co., Wood Dale, IL, USA) for automatic measurement of distance traveled in meters (both total and in the center area of the field), while grooming and rearing behaviors were manually scored using the Solomon Coder software (v17.03.22).

Gene expression analysis

Brains were quickly extracted on ice, and the DS, NAc, HPC, and mPFC were dissected as described elsewhere (Rojas-Carvajal *et al.,* 2020), following a right-and-left alternating method to ensure both hemispheres were equally represented in the analysis. Brain tissue samples were collected in a tube containing 300 µL of TRIzol (Invitrogen™, USA), homogenized by sonication for 20 seconds using an ultrasonic dismembrator (Fisher, USA), immediately frozen, and stored at −80 °C until analysis. Total RNA extraction followed the manufacturer's specifications. RNA was quantified using a Nanodrop® (Thermo, USA), and 500 ng of RNA was used for reverse transcription for cDNA synthesis, performed with the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA), followed by RT-qPCR using a Rotor-Gene Q (Qiagen, Germany). We measured the mRNA levels of BDNF, TrkB, CREB, CRF, and CRFR1. PCR conditions and primer validation were previously reported (Sequeira-Cordero *et al*., 2013). All the samples were run in duplicate, and the mean values were used for further calculations. Non-template controls and minus RT controls were included in each run. Fluorescence data were collected, and the threshold cycle (Ct) was calculated using the Rotor-Gene Q Series Software (QIAgen, Germany). Relative gene expression was determined by the comparative method with hypoxanthine phosphoribosyltransferase 1 (HPRT1) used as the reference gene. mRNA levels were reported as $2^{-\Delta Ct}$ mean values. For graphic representation, mRNA levels were expressed as percentages relative to the control group.

Western blot

Protein isolation from the DS, NAc and mPFC followed a modified procedure outlined by Kopec *et al.* (2017) using the TRIzol homogenates after RNA purification. After protein precipitation using the TRIzol method, the samples were homogenized in a lysis buffer (20 mM EDTA, 140 mM NaCl, 2% SDS, 100 mM Tris pH 8.0) supplemented with

protease inhibitors (Roche, #11836170001) and phosphatase (Thermo, #A32957) inhibitors. Then, samples were incubated at 50°C for 2 hours and centrifuged for 5 minutes at 7600 × *g* to collect the protein supernatants. All procedures were performed on ice. Protein concentration was determined using the Nanodrop® (Thermo, USA) with the BCA method, following the specified instructions (Thermo Micro BCA Protein Assay, #23235). Equal amounts of proteins were mixed with Laemmli buffer and heated at 95°C for 5 minutes. The proteins were loaded onto either 12% or 15% (only for CREB detection) SDS-PAGE gels and separated by electrophoresis at 120V for 2 hours. The proteins were then electrotransferred onto nitrocellulose membranes for 2 hours at 80V. After transfer, the membranes were blocked with 5% non-fat milk in Tris-buffered saline with 0.05% Tween 20 (TBST) at 4°C for 1 hour. Membranes were then incubated overnight at 4°C with primary antibodies for detecting BDNF (mature form), CREB, and its active form, pCREB. The following monoclonal antibodies were used: anti-BDNF (Abcam, #ab108319, 1:500), anti-CREB (Abcam, #ab32515, 1:2000), and anti-pCREB (Abcam, #ab32096, 1:1000). Rabbit polyclonal anti-HPRT (Abcam, #ab10479, 1:1500) was used as loading control. All primary antibodies were diluted in TBST. The next day, the membranes were washed seven times with TBST (each wash lasting 5 minutes) and incubated for 1 hour with goat-anti-rabbit IgG H&L-HRP (Abcam, #ab205718) as the secondary antibody, diluted in 3% non-fat milk in TBST. Finally, the membranes were treated with an electrochemiluminiscent reagent (Thermo, #32106) and visualized using the ChemiDoc Imaging System (Bio-Rad). Densitometry analysis for protein quantification was performed using Image Lab software (Bio-Rad). All proteins were normalized to the levels of HPRT, and pCREB was normalized to the total amount of CREB.

Statistical analysis

All statistical analyses were carried out using IBM® SPSS Statistics v25.0 software (IBM Corp., Armonk, NY, USA). Data are presented as the mean ± standard error of the mean (SEM). *P*-values < 0.05 were considered statistically significant, and the partial eta-squared coefficient (η_p^2) was reported as an index of effect size. Weekly body weight,

food, water, and alcohol intake were analyzed using a mixed two-way analysis of covariance (ANCOVA) with the Week as within factor, and Diet and Alcohol as the between-groups factors. In the case of Mauchly's sphericity violation, the Greenhouse-Geisser correction was applied. Cumulative comparisons of nutritional variables, water consumption, alcohol intake, biometric and behavioral parameters, as well as gene expression (mRNA and protein levels) were analyzed using a two-way multivariate covariance (MANCOVA) analysis. We included the dam of origin as a covariate to control for that biological factor, as previously stated (Sequeira-Cordero *et al*., 2013, 2019; Vindas-Smith *et al.,* 2022). Additionally, for gene expression analyses, we included the subjects as a variable to weigh up the MANCOVA analysis to control for the inter-individual variability that may exert a substantial impact on the estimation of the parameters and the statistical significance. Finally, we performed Pearson correlation analyses among body weight-related parameters, food intake variables, alcohol consumption, and behavioral parameters with mRNA and protein expression.

RESULTS

Food and macronutrient intake

Diet had a significant main effect on food (g) and energy (kcal) intake, while alcohol drinking had no effect in all food intake parameters tested (all *p*-values > 0.05). All rats experience a higher intake over the weeks (Week: $F_{1.27,57.21} = 11.99$, $p < 0.0001$, $\eta^2_p = 0.21$). Food intake differed between groups during all weeks except on week 6 (**Fig. 2**A), with cafeteria-fed rats consuming more food than the standard-fed counterparts (main Diet effect: $F_{1,45} = 24.35$, $p < 0.0001$, $\eta^2_p = 0.35$, **Fig. 2**B). Throughout the 9-week protocol, cafeteria-fed rats showed a significant increase in energy intake since week 1, which stabilized after week 6 (Week × Diet: $F_{5.23,235.46} = 15.33$, $p < 0.0001$, $\eta^2_p = 0.25$, **Fig. 2**C). In consequence, cumulative kcal intake was significantly higher in the cafeteria groups (main Diet effect: $F_{1,45} = 112.87$, $p < 0.0001$, $\eta^2_p = 0.72$, **Fig. 2**D).

As shown in **Fig.** 3A-E (left panels), there was a within-effect of Week on macronutrient intake (all *p*-values < 0.0001), with all animals increasing their consumption during the first four weeks and then stabilizing their intakes until the end of the protocol. A Week × Diet interaction was observed for all macronutrients (all *p*-values < 0.0001), with cafeteria-fed rats exhibiting a higher consumption of carbohydrates and fat, and a lower intake of proteins. Accordingly, these over-week differences were reflected in the cumulative intake of all macronutrients. Throughout the 9-week protocol, cafeteria-fed rats consumed more carbohydrates ($F_{1,45} = 45.98$, $p < 0.0001$, $\eta^2_p = 0.51$, **Fig. 3**A, right panel), fat ($F_{1,45} = 962.45$, $p < 0.0001$, $\eta^2_p = 0.96$, **Fig. 3**B, right panel), and less protein ($F_{1,45} = 385.30$, $p < 0.0001$, $\eta^2_p = 0.90$, **Fig. 3**C, right panel). Fat intake was the most affected macronutrient variable by the diet. Moreover, rats that fed the cafeteria diet ate less fiber ($F_{1,45} = 375.74$, $p < 0.0001$, $\eta^2_p = 0.89$) and more sugar ($F_{1,45} = 1768.87$, $p < 0.0001$, $\eta^2_p = 0.98$, **Fig. 3**E, right panel) than standard-fed rats. Sugar intake was the variable most influenced regarding total carbohydrate consumption.

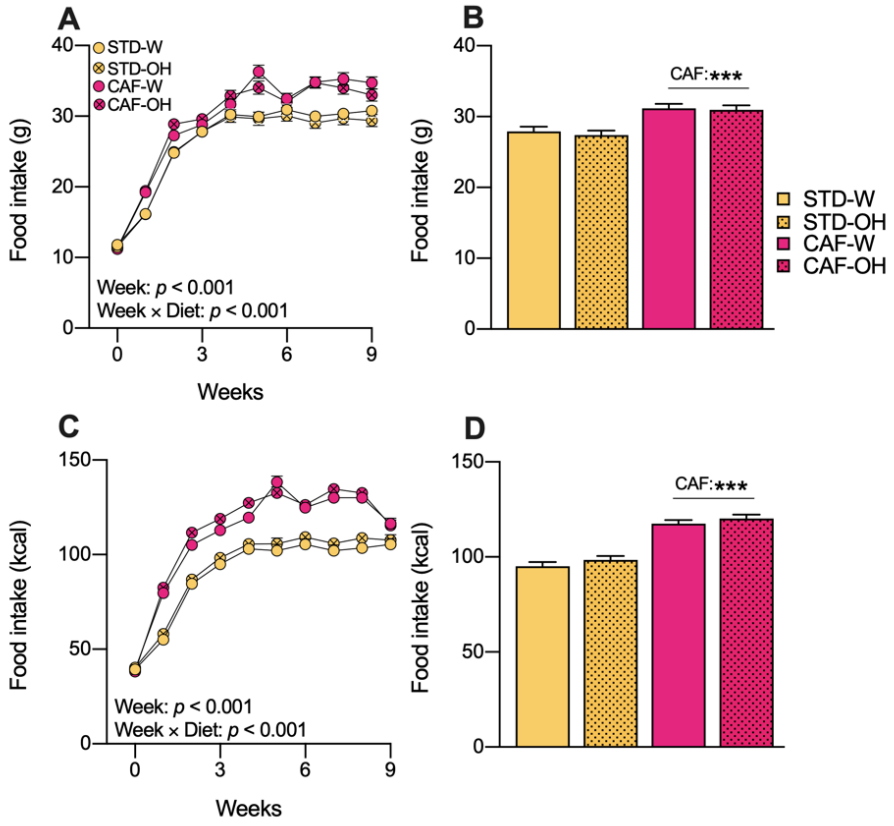

Figure 2. Food and energy intake during 9 weeks of exposure to the treatments. **(A)** Week-by-week measurements of food intake, and **(B)** mean cumulative food intake in grams after a 9-week

exposure. **(C)** Energy intake (kcal) per week, and **(D)** cumulative energy intake. Data are expressed as mean ± SEM. STD-W, control group; STD-OH, alcohol group; CAF-OH, cafeteria group; CAF-OH, cafeteria-alcohol group (n = 11-13 animals/group): *** p<0.001; W, water; OH, alcohol; STD, standard diet, CAF, cafeteria diet. See main text for details.

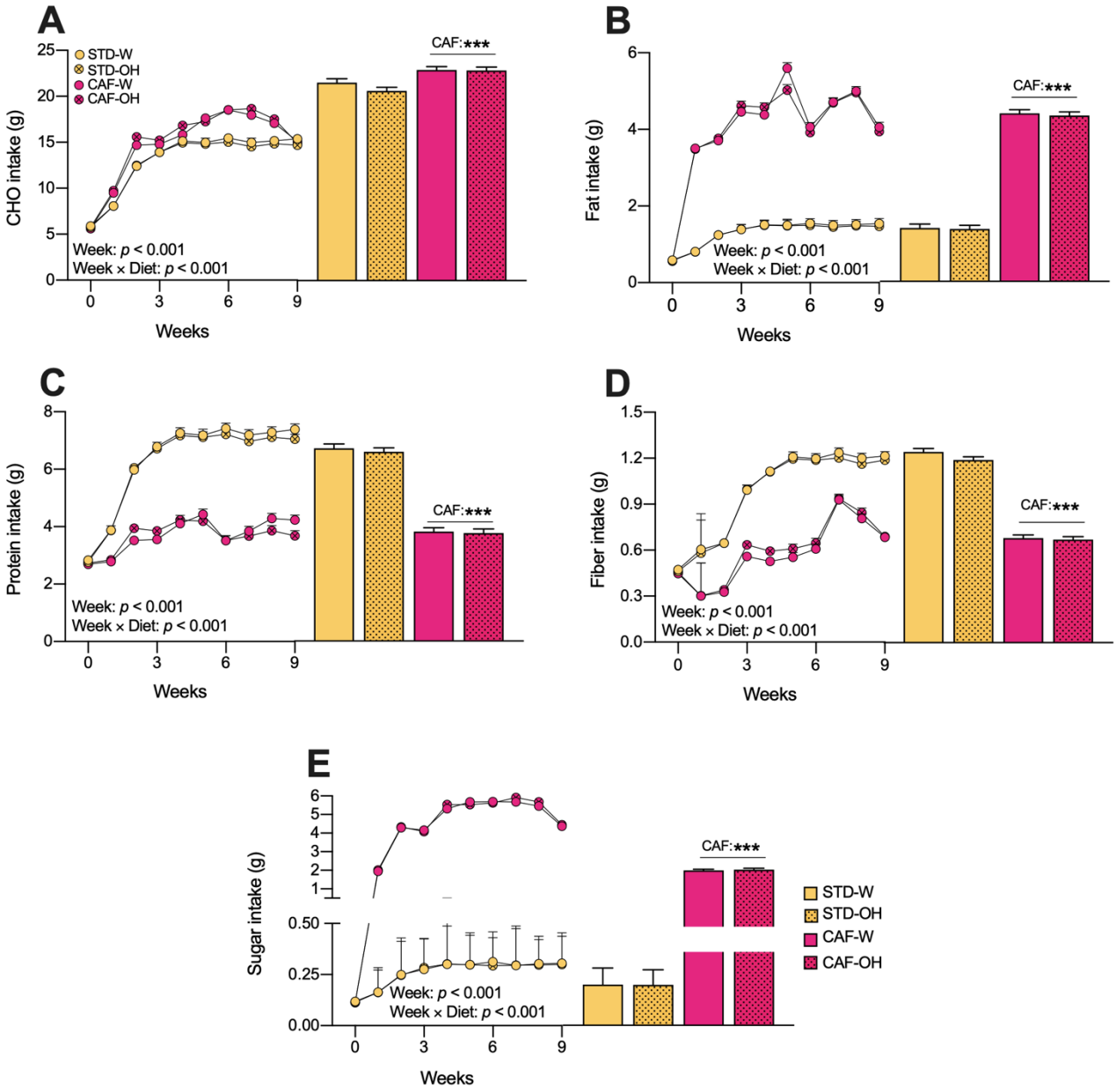

Figure 3. Macronutrient intake during 9 weeks of exposure to the cafeteria diet and alcohol drinking. Week-by-week measurements (left panels) and cumulative intake values (right panels) for **(A)** Carbohydrates, **(B)** Fat, **(C)** Protein, **(D)** Fiber, and **(E)** Sugar. Data are expressed as mean

± SEM. STD-W, control group; STD-OH, alcohol group; CAF-OH, cafeteria group; CAF-OH, cafeteria-alcohol group (n = 11-13 animals/group): *** p<0.001; W, water; OH, alcohol; STD, standard diet, CAF: cafeteria diet. See main text for details.

Alcohol and water consumption

All rats increased their water intake through the weeks (Week: $F_{3.05,137.39} = 26.08$, $p < 0.0001$, $\eta^2_p = 0.37$, **Fig. 4**A), but animals with access to alcohol showed a lesser increase (Week × Alcohol: $F_{3.05,137.39} = 8.05$, $p < 0.0001$, $\eta^2_p = 0.15$, **Fig. 4**A). A main effect of Diet ($F_{1,45} = 5.98$, $p = 0.018$, $\eta^2_p = 0.12$) and Alcohol ($F_{1,45} = 5.00$, $p = 0.03$, $\eta^2_p = 0.10$) was found in cumulative water intake (**Fig. 4**B). Cafeteria-fed rats, and animals with access to alcohol showed lower water intake relative to the control group. Regarding alcohol intake, all animals exposed to alcohol showed a scaling pattern in their consumption over the weeks (Week: $F_{3.32,76.26} = 5.61$, $p = 0.001$, $\eta^2_p = 0.20$, **Fig. 4**C), except during week 2. Standard-fed rats, however, displayed a more pronounced scaling pattern than cafeteria-fed rats throughout the testing period (Week × Diet: $F_{3.32,76.26} = 5.97$, $p = 0.0007$, $\eta^2_p = 0.21$, **Fig. 4**C). Consequently, cumulative alcohol intake was significantly lower in cafeteria-fed rats compared to their standard-fed counterparts (main Diet effect: $F_{1,23} = 15.04$, $p = 0.0008$, $\eta^2_p = 0.40$, **Fig. 4**D). Alcohol preference relative to total liquid intake was also lower in cafeteria-fed rats (main Diet effect: $F_{1,23} = 10.19$, $p = 0.004$, $\eta^2_p = 0.31$, **Fig. 4**E.

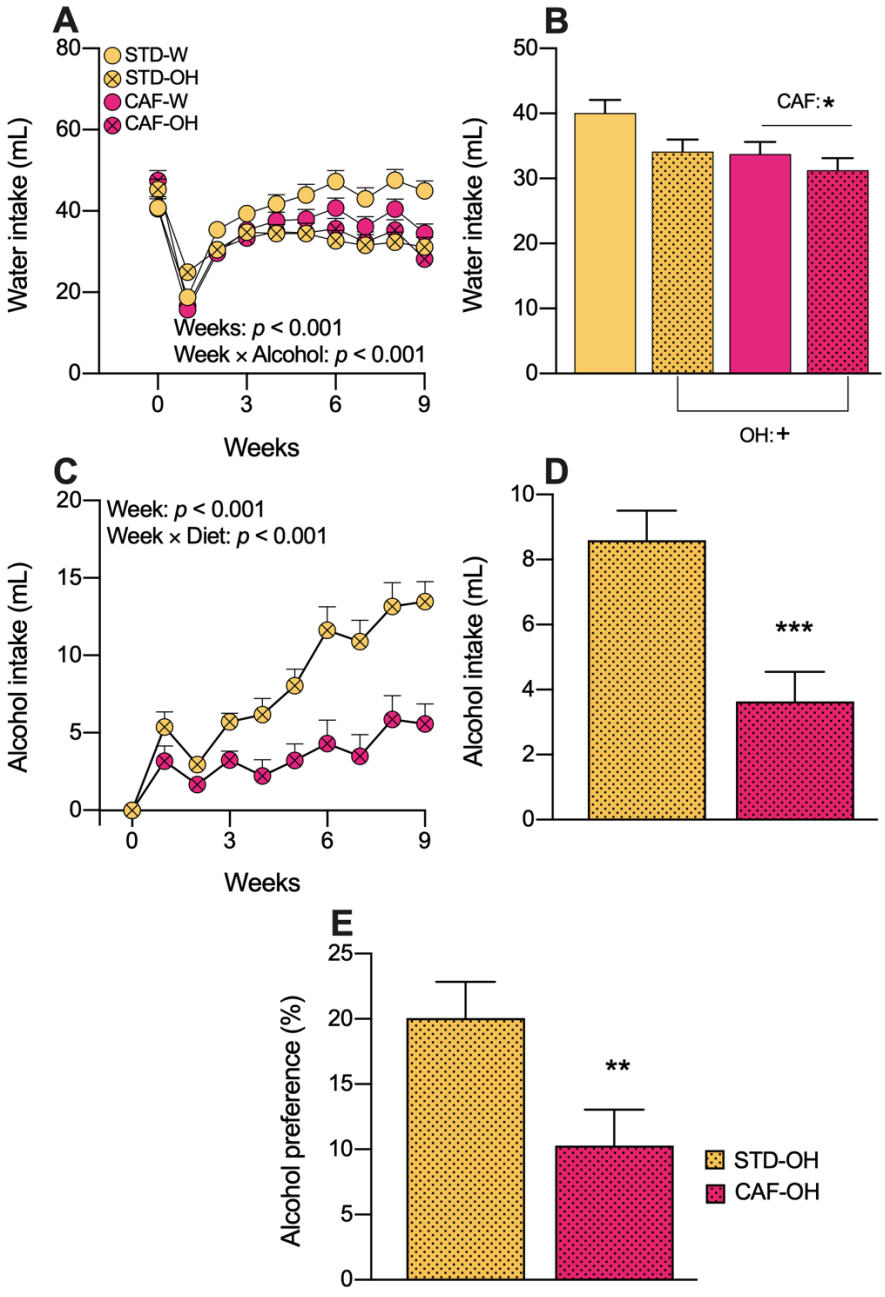

Figure 4. Alcohol and water intake during 9 weeks of exposure to the treatments. **(A)** Week-by-week measurements of water intake (mL). **(B)** Cumulative water intake. **(C)** Week-by-week measurements of alcohol intake (mL). **(D)** Cumulative alcohol intake. **(E)** Alcohol preference expressed as percentage of total liquid intake. Data are expressed as mean ± SEM. STD-W, control group; STD-OH, alcohol group; CAF-OH, cafeteria group; CAF-OH, cafeteria-alcohol group (*n* = 11-13 animals/group): * $p<0.05$, ** $p<0.01$, *** $p<0.001$; W, water; OH, alcohol; STD, standard diet, CAF, cafeteria diet. See main text for details.

Biometric parameters

As expected for young animals, there was an effect of Week on body weight with all animals exhibiting an increasing growth rate ($F_{1.17,52.49} = 998.03$, $p < 0.0001$, $\eta^2_p = 0.96$, **Fig. 5**A). The cafeteria-fed animals gained more weight over time than controls (Week × Diet: $F_{1.17,52.49} = 45.33$, $p < 0.0001$, $\eta^2_p = 0.50$, **Fig. 5**A), with significant between-group differences being observed from the 3rd week onward. Consequently, a main effect of the cafeteria diet on final body weight ($F_{1,45} = 41.06$, $p < 0.0001$, $\eta^2_p = 0.48$, **Fig. 5**B) and the percentage of body weight gain was found ($F_{1,45} = 51.17$, $p < 0.0001$, $\eta^2_p = 0.53$, **Fig. 5**C). Regarding body fat accumulation, the cafeteria diet increased perigonadal ($F_{1,45} = 48.29$, $p < 0.0001$, $\eta^2_p = 0.52$, **Fig. 5**D), abdominal ($F_{1,45} = 45.96$, $p < 0.0001$, $\eta^2_p = 0.51$, **Fig. 5**D), and total adipose tissue ($F_{1,45} = 83.28$, $p < 0.0001$, $\eta^2_p = 0.65$, **Fig. 5**D). Alcohol, irrespective of the diet, also increased the percentage of abdominal (main Alcohol effect: $F_{1,45} = 5.43$, $p < 0.024$, $\eta^2_p = 0.11$, **Fig. 5**D) and total body fat (main Alcohol effect: $F_{1,45} = 4.57$, $p < 0.038$, $\eta^2_p = 0.09$, **Fig. 5**D). There were no interactions between both factors.

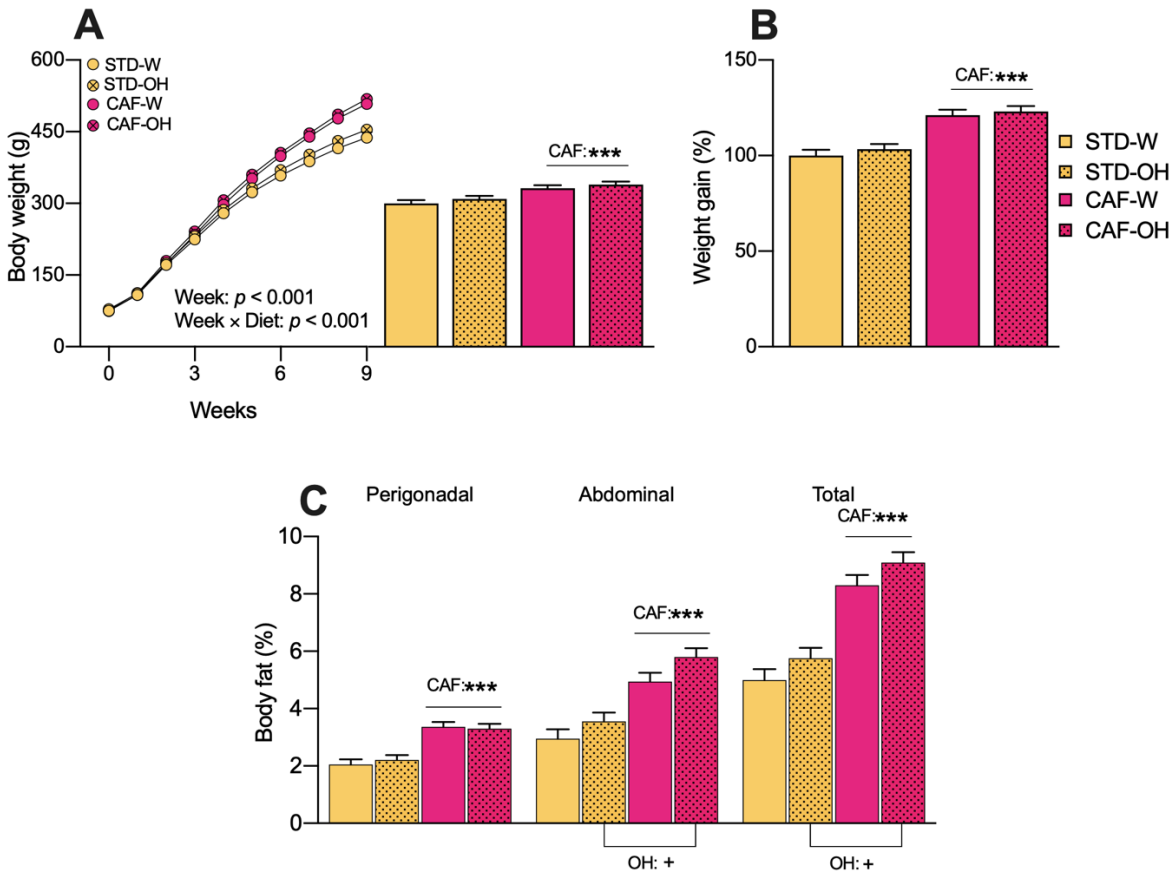

Figure 5. Obesity-related parameters in rats exposed to a 9-week cafeteria diet and alcohol intake. **(A)** Weekly body weight. **(B)** Final body weight after 9-week exposure to the treatments. **(C)** Body weight gain expressed as a percentage relative to the control group. **(D)** Body fat mass accumulation for perigonadal, abdominal, and total white adipose tissues expressed as a percentage of body weight at euthanasia. Data are expressed as mean ± SEM. STD-W, control group; STD-OH, alcohol group; CAF-OH, cafeteria group; CAF-OH, cafeteria-alcohol group (*n* = 11-13 animals/group): *** $p<0.001$, + $p<0.05$; W, water; OH, alcohol; STD, standard diet, CAF, cafeteria diet. See main text for details.

Behavioral characterization in the open field test

As compared with standard-fed rats, cafeteria-fed counterparts traveled less distance ($F_{1,91}$ = 4.14, p = 0.045, η^2_p= 0.04, **Fig. 6**A) and displayed fewer rearing events (time: $F_{1,91}$ = 6.91, p = 0.01, η^2_p= 0.07, **Fig. 6**B; frequency: $F_{1,91}$ = 4.94, p = 0.029, η^2_p = 0.05, **Fig. 6**C), while did more grooming (time: $F_{1,91}$ = 10.35, p = 0.002, η^2_p = 0.10, **Fig. 6**D; frequency:

$F_{1,91} = 6.03$, $p = 0.016$, $\eta^2_p = 0.06$, **Fig. 6**E). In addition, alcohol-provided rats traveled less distance (main Alcohol effect: $F_{1,91} = 4.34$, $p = 0.039$, $\eta^2_p = 0.046$, **Fig. 6**A). Regarding the habituation effect, only rearing behavior showed a significant decrease from day 1 to 2 in all animals regardless of the treatment received ($F_{1,91} = 19.62$, $p < 0.001$, $\eta^2_p = 0.12$, **Fig. 6**C).

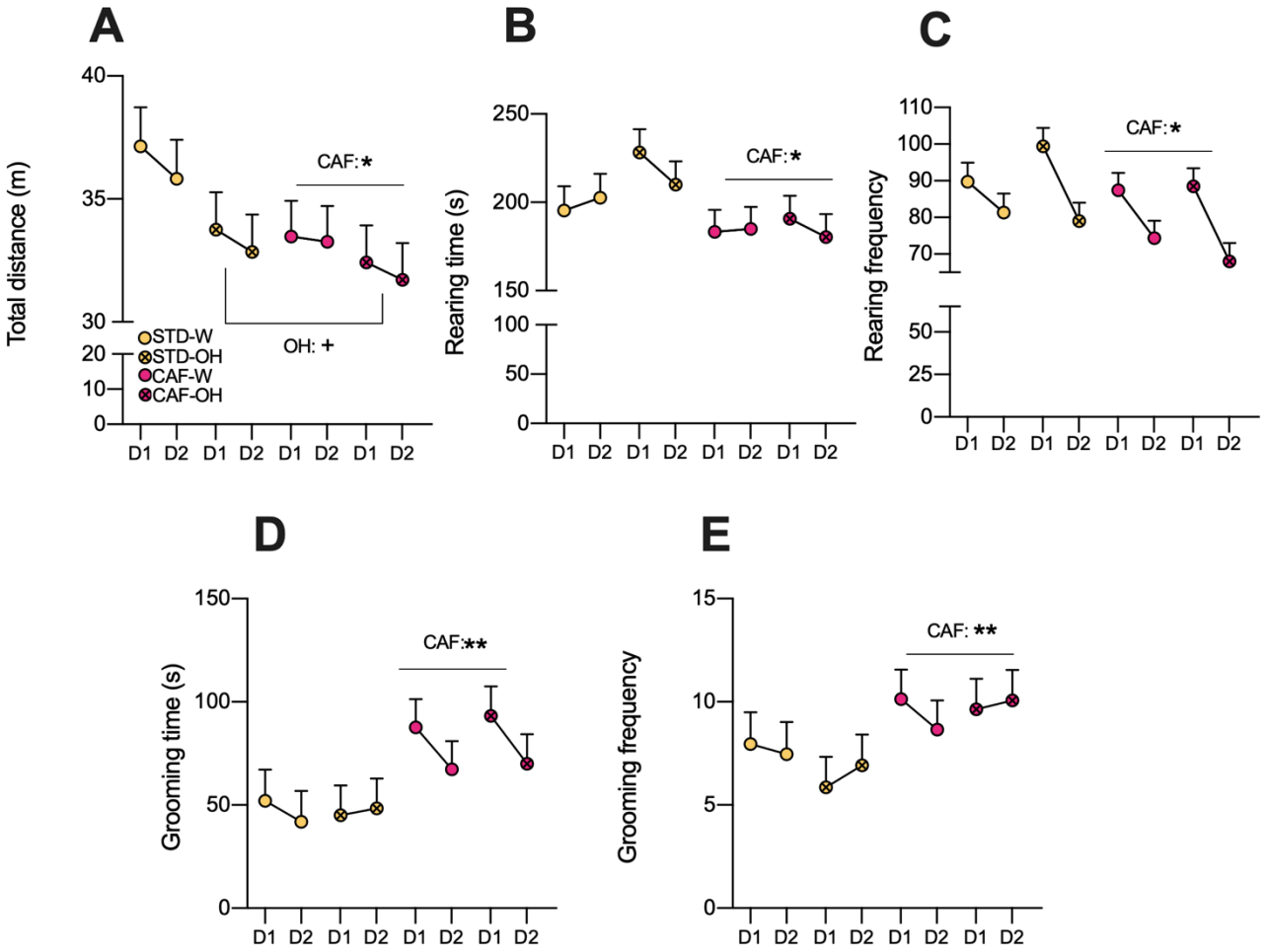

Figure 6. Behavioral assessment in the open field test after 9 weeks of exposure to the treatments. **(A**) Total distance traveled in meters. **(B)** Total rearing time in seconds. **(C)** Total rearing frequency. **(D)** Total grooming time **(E)** Total grooming frequency. Figures are represented despite significative variables those effects were significative. Data are expressed as mean ± SEM. STD-W, control group; STD-OH, alcohol group; CAF-OH, cafeteria group; CAF-OH, cafeteria-alcohol group (n = 11-13 animals/group): ** p<0.001, * p<0.01, + p<0.05; W, water; OH, alcohol; STD, standard diet, CAF, cafeteria diet; D1, day 1; D2, day 2. See main text for details.

Gene expression analyses in brain reward-related regions

mRNA levels

There were no significant effects of Diet and Alcohol in accumbal and dorsal striatal mRNA levels. Cafeteria-fed rats showed lower and higher levels of CREB in the mPFC ($F_{1,45}$ = 5.21, p = 0.027, η^2_p = 0.10, **Fig. 7**A) and the HPC ($F_{1,45}$ = 4.96, p = 0.031, η^2_p = 0.099, **Fig. 7**B), respectively. Alcohol-provided rats, on the other hand, showed lower levels of TrkB ($F_{1,45}$ = 4.79, p = 0.034, η^2_p = 0.096, **Fig. 7**B), CREB ($F_{1,45}$ = 9.65, p = 0.003, η^2_p = 0.18, **Fig. 7**B) and CRFR1 ($F_{1,45}$ = 5.06, p = 0.029, η^2_p = 0.12, **Fig. 7**B) in the HPC. Finally, there was a significant Diet × Alcohol interaction effect on the expression of CRFR1 in the mPFC ($F_{1,45}$ = 5.06, p = 0.029, η^2_p = 0.10, **Fig. 7**A), with the cafeteria-alcohol rats showing the highest mRNA levels compared with the rats exposed either to alcohol or the cafeteria diet.

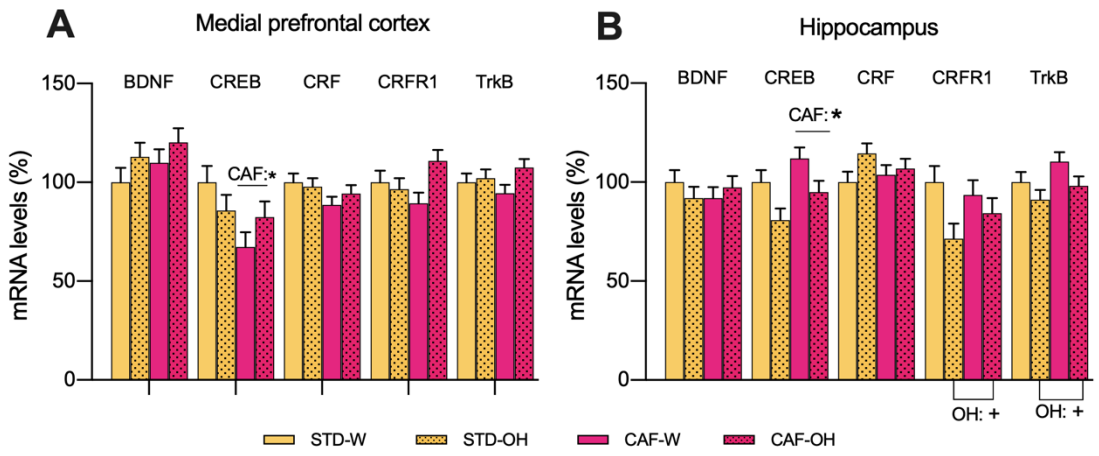

Figure 7. mRNA levels in the **(A)** medial prefrontal cortex and **(B)** the hippocampus of rats exposed either to standard or cafeteria diet, and with voluntary access to alcohol. Gene expression is expressed as percentage relative to the control group. Data are expressed as mean ± SEM. BDNF, brain-derived neurotrophic factor; CREB, cAMP response element-binding protein; CRF, corticotropin-releasing factor, CRFR1, CRF receptor type 1; TrkB, tropomyosin receptor kinase B; STD-W, control group; STD-OH, alcohol group; CAF-OH, cafeteria group; CAF-OH, cafeteria-alcohol group (n = 11-13 animals/group): * p<0.05, + p<0.05; W, water; OH, alcohol; STD, standard diet, CAF, cafeteria diet See main text for details.

Protein expression levels

No main effects of Diet and Alcohol were detected for the protein levels in the mPFC, the DS, and the NAc. However, there was a significant Diet × Alcohol interaction effect for pCREB/CREB in the mPFC ($F_{1,45}$ = 4.91, p = 0.032, η^2_p = 0.098, **Fig. 8**B), with the cafeteria-alcohol group showing the lowest levels compared to the rats exposed to either the cafeteria diet or alcohol. In the DS, a significant Diet × Alcohol interaction was also detected for the levels of CREB ($F_{1,45}$ = 7.93, p = 0.007, η^2_p =0.15, **Fig. 8**C) and pCREB/CREB ($F_{1,45}$ = 8.25, p = 0.006, η^2_p = 0.16, **Fig. 8**C). In both cases, the standard-water group had higher and lower protein levels than the other groups, respectively.

Correlation analyses

Regarding the correlation analyses among food intake, biometric parameters, mRNA and protein levels, there was a significant positive association between abdominal fat and the mRNA levels of BDNF and TrkB in the mPFC in the cafeteria-fed rats (r = 0.458, p = 0.019 and r = 0.404, p = 0.041, respectively). Moreover, body weight gain negatively correlated with the levels of CRF mRNA in the HPC (r = -0.402, p = 0.042), and pCREB (active protein) in the mPFC and NAc (r = -0.525, p = 0.006; r = -0.454, p = 0.020, respectively). The expression of CRF in the HPC was negatively correlated with body weight gain in the cafeteria-alcohol group (r= -0.565, p = 0.044). In addition, in alcohol-provided rats, accumbal BDNF mRNA levels were negatively correlated with the BDNF protein levels in the same region (r = -0.613, p = 0.023). Also, in the mPFC, CREB protein expression was negatively correlated with its mRNA levels (r = -0.553, p = 0.049). In both groups with alcohol access, there was a positive correlation between pCREB and CREB protein levels in the DS (standard-alcohol group: r = 0.715, p = 0.006; cafeteria-alcohol group: r = 0.896, p < 0.001). Finally, a positive correlation between CREB protein levels and its mRNA levels in the DS was found (r = 0.733, p = 0.004) in the cafeteria-alcohol group.

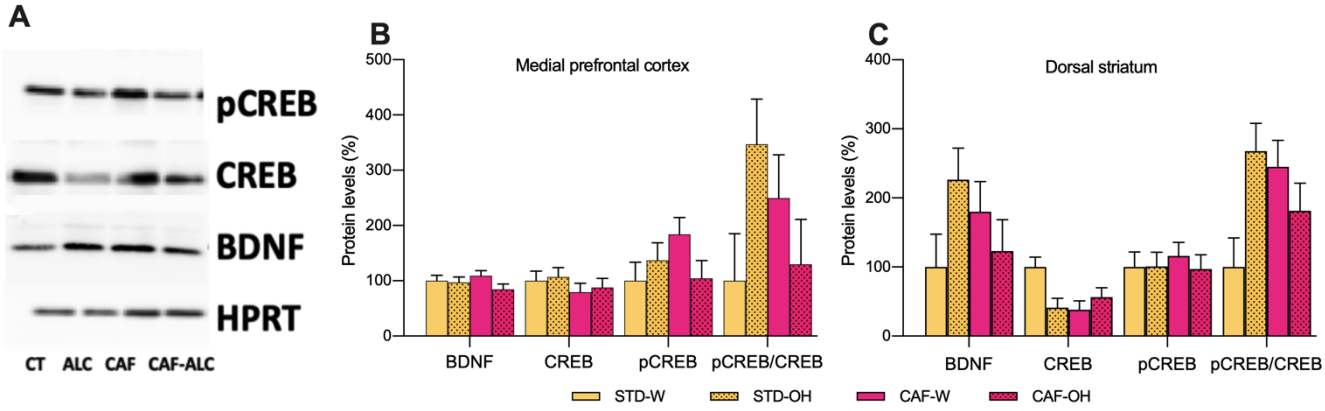

Figure 8. Protein levels in the medial prefrontal cortex and the dorsal striatum of rats exposed either to standard or cafeteria diet, and with voluntary access to alcohol. **(A)** Example of a Western blot with the analyzed proteins in the DS. **(B)** Protein levels in the medial prefrontal cortex and the **(C)** dorsal striatum. HPRT was used as loading control. Protein levels are expressed as percentage relative to the control group. Data are presented as mean ± SEM. BDNF, brain-derived neurotrophic factor; CREB, cAMP response element-binding protein; pCREB, phosphorylated form of CREB; pCREB/CREB; ratio of pCREB and CREB levels; STD-W, control group; STD-OH, alcohol group; CAF-OH, cafeteria group; CAF-OH, cafeteria-alcohol group (n = 11-13 animals/group); W, water; OH, alcohol; STD, standard diet, CAF, cafeteria diet.

DISCUSSION

We implemented a slightly modified version of our previously published cafeteria diet protocol (Vindas-Smith *et al*., 2022) by including free-voluntary alcohol consumption to study the independent and combined effects of cafeteria diet and alcohol on biometric, neurochemical (i.e., gene and protein expression), and behavioral parameters in juvenile, male Wistar rats. The cafeteria diet model consists of providing laboratory rats with flavorsome and unhealthy food products commonly consumed by humans in Western countries (e.g., cookies, sausages, sugar-sweetened beverages). Based on previous experiments, we selected the rats' most-preferred food items that also suffer the minimal degradation of their organoleptic properties (i.e., taste, smell, and texture). To maximize food consumption, the food items were presented in different combinations –to the largest extent possible– to avoid sensorial habituation and maintain dietary variation.

Presumably, hyperphagia resulted from the high palatability and caloric content of the cafeteria diet.

We found that exposure to the cafeteria diet for nine weeks led to increased food intake, body weight gain, and fat accumulation, especially in the abdominal area. The combination of these three mutually related parameters –which constitute the gold standard of obesity– was successfully modified by our diet protocol. Alcohol on its own did not affect food intake and body weight gain. Rats that were daily exposed to alcohol as a continuous treatment had two bottles, one with water and one with alcohol. Thus, alcohol consumption was voluntary and somewhat competed with water and food intake. For instance, alcohol-exposed rats fed with the cafeteria diet drank less alcohol. This in agreement with a long-lasting reduction in 2-hour ethanol intake in rats previously exposed a cafeteria diet (Cook *et al.*, 2017), and with the observation that mice fed with a high-fat diet showed reduced preference for ethanol in a 24-hour test (Takase *et al.*, 2017). In those rats exposed to alcohol and cafeteria diet simultaneously, the reduced alcohol drinking could have been a compensatory response for consuming a high caloric diet (Cook *et al.*, 2017). Alternatively, the rewarding properties of the cafeteria diet could have outpaced those of alcohol. First, because the diet has a higher caloric content while being more appealing as a primary reinforcer. Second, the alcohol solution was prepared with no flavorings and probably was less tasty than the foods composing the cafeteria diet. Third, the cafeteria diet could have produced a cross-tolerance like effect to the rewarding action of alcohol in the mesolimbic reward system. In that regard, chronic consumption of a cafeteria diet has been found to reduced sucrose intake in the sucrose preference test, supporting the hypothesis that the cafeteria diet blunts the hedonic value of otherwise highly rewarding stimuli (Gonçalves *et al.*, 2021; Alvarez-Monell *et al*., 2022).

Although not all animals preferred and then consumed alcohol sufficiently to affect body weight, it was enough to increase the fat accumulation in the abdominal area irrespective of the diet. High body fat content, especially in the abdominal region, has previously been observed in animals fed with a cafeteria diet (Lewis *et al.*, 2019) and those exposed to alcohol (Teixeira *et al.*, 2020). Even though it has been recognized that factors such as

alcohol preference, genetics, gender, and age interact to determine the accumulation of central-body and abdominal fat (Ferreira *et al.*, 2008), the amount of alcohol consumed daily plays a critical role. In alcohol drinkers, the higher the alcohol intake, the higher the body weight and fat accumulation both in the neck and waist (Brenes *et al.*, 2021), which was not attributable to caloric sources other than alcohol. It is worth noting that the cafeteria diet induced effect sizes in abdominal fat accumulation that were four times larger than those induced by alcohol. However, the combined impact of these two factors on visceral fat depots is a significant health concern, given that consuming alcoholic beverages before, during, or after calorie-dense meals is a common practice, particularly during social events (Caton *et al.*, 2015). It is well known that the accumulation of abdominal fat is associated with a higher risk of obesity-related complications, including metabolic syndrome and cardiovascular diseases (Calabro *et al.*, 2007).

Regarding the consumption of macronutrients, cafeteria-fed rats consumed more carbohydrates, fat, and sugar and less protein and fiber. This consumption pattern may be attributable to the nutritional characteristics of highly processed foods, which tend to have lower protein and higher fat contents. A lower protein intake has been found to promote hyperphagia, especially from high-fat and high-sugar food to compensate for the decreased energy intake from proteins, a phenomenon described as protein leverage hypothesis (Raubenheimer & Simpson, 2023). Concomitantly, chronic fat ingestion is known to reduce sensitivity to satiation-feedback signals from the gastrointestinal tract (Smith & Moran, 2021). These two factors interact synergistically to disrupt appetite regulation, contributing to the development of obesity (Steele *et al.*, 2018). As expected, food intake variables showed very large effect sizes, with fat and sugar intake being the most affected by the cafeteria diet, explaining 96% and 98% of the variance, respectively.

At the behavioral level, both cafeteria-fed and alcohol-provided rats displayed lower exploratory OFT activity. In rodents, the open and illuminated OFT space elicits risk-assessment and defensive behaviors aimed at preventing being preyed (Blanchard *et al.*, 2003). Thus, increased activity in the OFT is interpreted as a behavioral marker of unconditioned anxiety (Blanchard *et al.*, 2003; Sturman *et al.*, 2018). In this line, the

cafeteria diet also increased grooming behavior. Although grooming behavior in rodents has been associated with emotional distress, cumulative evidence indicates that OFT grooming is a reliable proxy of the activation of an arousal-inhibiting system serving to restore emotional homeostasis, so that the higher the grooming, the lower the unconditioned anxiety levels (Delius, 1967; Brenes *et al*., 2009; Rojas-Carvajal *et al*., 2020; 2022). Finally, we performed two consecutive OFT tests to assess whether the cafeteria diet or the alcohol intake affects habituation. Habituation is a non-associative learning process inferred from a reduction in OFT parameters after repeated exposures (Rojas-Carvajal *et al*., 2020; 2022). Rearing was the only behavior that showed a habituation effect, with no between-group differences. Thus, the lower OFT activity induced by the treatments may have resulted from a diminished behavioral reactivity to the mild stress of the OFT and not from a faster habituation process. Altogether, the behavioral data suggest that the cafeteria diet and, to a lesser extent the alcohol intake, induced a mild anxiolytic-like effect without altering general psychomotor activity. These findings are agreement with a large body of evidence from human studies about emotional eating and the tranquilizer effect of highly palatable foods (Tomiyama *et al.*, 2011).

Cafeteria diet downregulated CREB expression in the mPFC. The cafeteria diet also reduced the levels of CREB protein in the DS. In the mPFC, this treatment did not change CREB and pCREB levels. Nevertheless, the cafeteria diet increased the ratio of pCREB/CREB in the mPFC and the DS. This indicates that the activated form of CREB was predominant relative to its non-active form, suggesting that the rate of gene transcription was augmented in those regions. Thus, low CREB mRNA levels could have resulted from a compensatory response to higher availability of its activated form, as suggested by the negative relationship found between CREB protein and mRNA levels. Signal transduction of CREB-related ligands –like BDNF– could have triggered its phosphorylation. In this line, our correlation analysis showed that rats with higher abdominal fat overexpressed BNDF and TrkB in the mPFC. The upregulation of BDNF in that region has been observed in animals with restricted access to the cafeteria diet (Virtuoso *et al.*, 2022). We also found that rats with higher body weight gain had lower levels of pCREB protein in the mPFC and NAc. In that regard, CREB downregulation in

mPFC has been linked to hyperphagia due to an impairment in the self-control of the eating behavior (Wang *et al*., 2020).

In the HPC, on the contrary, CREB was upregulated in cafeteria-fed rats, in agreement with our previous report (Vindas-Smith *et al*., 2022). Our correlation analysis also showed that rats with higher body weight gain had lower levels of CRF mRNA in the HPC. Our data suggest that the cafeteria diet may have initiated neuroplastic changes mediated by the recruitment of BDNF-related effectors such as CREB. By increasing or reducing the levels of this transcriptional factor, the cafeteria diet could have regulated gene expression related to hyperphagia in a region-dependent manner. In support of the latter, it has been found that exposure to a cafeteria diet in adolescents, but not in young adult mice, increased the number of dendritic spines and the grey matter in the CA1 area of the HPC (Valladolid-Acebes *et al.*, 2013; Sack *et al*., 2017), which constitute markers of enhanced structural plasticity.

Rats exposed to alcohol showed lower TrkB and CREB mRNA levels in the HPC. Repeated alcohol exposure is known to upregulate NMDA glutamate receptors and excitatory signaling, which increases the risk of Ca^{2+} excitotoxicity (Chandrasekar, 2013). In that regard, we found that alcohol increased the pCREB/CREB ratio in mPFC and DS, which could have resulted from more NMDA receptors available, as activation of NMDA receptors is known to increase the phosphorylation of CREB through Ca^{2+}/CaMK-dependent pathways (Wang *et al*., 2018). Thus, reduced CREB protein contents in DS and CREB and TrkB mRNA levels in HPC would indicate a reduction of BDNF-dependent plasticity to promote neuronal shrinkage as a cytoprotective, compensatory mechanism to prevent cell injury and death, as suggested elsewhere (Motaghinejad *et al.*, 2020). Lower levels of CREB mRNA have also been observed after repeated amphetamine exposure, especially in highly sensitized rats (Sequeira-Cordero & Brenes, 2021). On the other hand, we found that alcohol downregulated the expression of CRFR1 in the HPC and mPFC. Chronic alcohol consumption is known to increase glucocorticoids (GC) levels, which lead to two synergic actions worsening the alcohol-induced excitotoxicity: the elevation of extracellular glutamate concentrations and the increase of NMDA receptors (Kamal *et*

al., 2020). So, the reduction in CRFR1 expression would constitute an additional compensatory action to prevent further neural damage, as CRFR1 signaling at the hypothalamic-pituitary axis triggers the release of GC. Alternatively, lower CRFR1 mRNA levels may have underlain membrane receptor downregulation due to excessive CRF availability. A large body of preclinical evidence indicates that alcohol-induced neuroadaptations mediating the stress response are related to higher CRF signaling (Pomrenze *et al*., 2017).

CONCLUSIONS

The present experiment aimed to study the independent and combined effects of a cafeteria diet and voluntary alcohol consumption on biometric, neurochemical (i.e., gene and protein expression), and behavioral parameters in juvenile male Wistar rats. In conclusion, the cafeteria diet outperformed the effects of alcohol exposure in most biometric, nutritional, and behavioral parameters. The cafeteria diet caused hyperphagia, body weight gain, and higher fat accumulation, both in the abdominal and perigonadal areas. Alcohol only increased abdominal fat, without affecting the other biometric parameters. At the behavioral level, the cafeteria diet, and to a lesser extent alcohol, induced an anxiolytic-like effect without affecting general psychomotor activity. At the brain level, however, the cafeteria diet and alcohol exerted independent and interactive actions on gene expression and protein levels. The cafeteria diet changed CREB expression in opposite directions between the mPFC and the HPC. Alcohol, in contrast, downregulated CREB, CRFR1, and TrkB expression in the HPC. Regarding protein contents, both treatments equally reduced CREB protein in the DS. However, alcohol, and to a lesser extent the cafeteria diet, increased the pCREB/CREB ratio in the mPFC and the DS. Considering the differences observed in mRNA and protein levels, CREB, and in second place CRFR1, were the most sensitive parameters to both treatments. Altogether, these findings suggest that our model is useful and reliable for studying the neurobehavioral effects of a modern Westernized diet on the risk of suffering metabolic disorders and obesity.

REFERENCES

Alvarez-Monell, A., Subias-Gusils, A., Mariné-Casadó, R., Boqué, N., Caimari, A., Solanas, M., & Escorihuela, R. M. (2022). Impact of Calorie-Restricted Cafeteria Diet and Treadmill Exercise on Sweet Taste in Diet-Induced Obese Female and Male Rats. Nutrients, 15(1), 144.

Berthoud, H.R., Lenard, N.R., Shin, A.C. (2001). Food reward, hyperphagia, and obesity. *Am J PhysiolRegulIntegr Comp Physiol*, 300, R1266–R1277.

Blanchard, D.C., Griebel, G., Blanchard, R.J. (2003) The Mouse Defense Test Battery: pharmacological and behavioral assays for anxiety and panic. *Eur J Pharmacol* 463:97–116.

Blüher, M. (2020). Metabolically healthy obesity. *Endocrine reviews*, *41*(3): 1-16.

Blumenthal, D. M., & Gold, M. S. (2010). Neurobiology of food addiction. *Current Opinion in Clinical Nutrition & Metabolic Care*, *13*(4), 359-365.

Brenes, J.C., Nogueira Previdelli, Á., Fisberg, R. M., Kovalskys, I., Fisberg, M., Herrera-Cuenca, M., Cortés Sanabria, L. Y., Yépez García, M. C., Rigotti, A., Liria-Domínguez, M. R., Guajardo, V., Gómez, G. (2021). Alcohol contribution to total energy intake and its association with nutritional status in eight Latin American countries. *International Journal of Environmental Research and Public Health, 18,* 13130.

Brenes, J.C., Padilla, M. & Fornaguera, J. (2009). A detailed analysis of open-field habituation and behavioral and neurochemical antidepressant-like effects in postweaning enriched rats. *Behavioural Brain Research*, 197: 125-137.

Brutman, J., Davis, J. F., & Sirohi, S. (2020). Behavioral and neurobiological consequences of hedonic feeding on alcohol drinking. *Current pharmaceutical design*, *26*(20), 2309-2315.

Calabro, P., & Yeh, E. T. (2007). Intra-abdominal adiposity, inflammation, and cardiovascular risk: New insight in the global cardiometabolic risk. Current Cardiovascular Risk Reports, 1(1), 32-38.

Caton, S. J., Nolan, L. J., & Hetherington, M. M. (2015). Alcohol, appetite and loss of restraint. *Current obesity reports*, *4*, 99-105.

Chandrasekar, R. (2013). Alcohol and NMDA receptor: current research and future direction. *Frontiers in molecular neuroscience*, *6*, 14.

Cook, J. B., Hendrickson, L. M., Garwood, G. M., Toungate, K. M., Nania, C. V., & Morikawa, H. (2017). Junk food diet-induced obesity increases D2 receptor autoinhibition in the ventral tegmental area and reduces ethanol drinking. PLoS One, 12(8), e0183685.

Cordeira, J., & Rios, M. (2011). Weighing in the role of BDNF in the central control of eating behavior. *Molecular neurobiology*, *44*, 441-448.

De Castro, J. M. (2000). Eating behavior: lessons from the real world of humans. *Nutrition*, *16*(10), 800-813.

Delius, J. D. (1967). Displacement activities and arousal. *Nature*, *214*(5094), 1259-1260.

Ferreira, M. G., Valente, J. G., Gonçalves-Silva, R. M. V., & Sichieri, R. (2008). Alcohol consumption and abdominal fat in blood donors. *Revista de Saúde Pública*, *42*, 1067-1073.

Gazdzinski, S., & Durazzo, T. C. (2013). Alcohol use and abuse: effects on body weight and body composition. *Alcohol, Nutrition, and Health Consequences*, 89-96.

Gonçalves, J. S., Rocha-Gomes, A., Teixeira, A. E., da Silva, A. A., Lessa, M. R., Dessimoni-Pinto, N. A. V., ... & Riul, T. R. (2021). Cafeteria diet decreases sucrose preference and increases the sensitivity of risperidone in the caloric intake of Wistar rats. Nutrition & Food Science, 52(2), 270-280.

Iemolo, A., Blasio, A., St Cyr, S. A., Jiang, F., Rice, K. C., Sabino, V., & Cottone, P. (2013). CRF–CRF1 receptor system in the central and basolateral nuclei of the amygdala differentially mediates excessive eating of palatable food. *Neuropsychopharmacology*, *38*(12), 2456-2466.

Johnson, F., & Wardle, J. (2014). Variety, palatability, and obesity. *Advances in nutrition*, *5*(6), 851-859.

Kamal H, Tan GC, Ibrahim SF, Shaikh MF, Mohamed IN, Mohamed RMP, Hamid AA, Ugusman A and Kumar J (2020). Alcohol Use Disorder, Neurodegeneration, Alzheimer's and Parkinson's Disease: Interplay Between Oxidative Stress, Neuroimmune Response and Excitotoxicity. Front. Cell. Neurosci. 14:282.

Koob, G. F., & Volkow, N. D. (2010). Neurocircuitry of addiction. *Neuropsychopharmacology*, *35*(1), 217-238.

Kopec, A. M., Rivera, P. D., Lacagnina, M. J., Hanamsagar, R., & Bilbo, S. D. (2017). Optimized solubilization of TRIzol-precipitated protein permits Western blotting analysis to maximize data available from brain tissue. *Journal of neuroscience methods*, *280*, 64-76.

Kovalskys, I. Fisberg, M., Gómez, G. Pareja, R. Yépez García, M., Cortés Sanabria, L., Koletzko, B. (2018). Energy intake and food sources of eight Latin American countries: results from the Latin American Study of Nutrition and Health (ELANS). *Public Health Nutrition*, 21(14), 2535-2547.

Lewis, A. R., Singh, S., & Youssef, F. F. (2019). Cafeteria-diet-induced obesity results in impaired cognitive functioning in a rodent model. *Heliyon*, *5*(3).

McClung, C. A., & Nestler, E. J. (2008). Neuroplasticity mediated by altered gene expression. *Neuropsychopharmacology*, *33*(1), 3-17.

Morris, M.J., Beilharz, J.E., Maniam, J., Reichelt, A.C., Westbrook, R.F. (2015). Why is obesity such a problem in the 21st century? The intersection of palatable food, cues and reward pathways, stress, and cognition. *NeurosciBiobehav Rev*, 58, 36–45.

Motaghinejad, M., Mashayekh, R., Motevalian, M., & Safari, S. (2021). The possible role of CREB-BDNF signaling pathway in neuroprotective effects of minocycline against alcohol-induced neurodegeneration: molecular and behavioral evidence. *Fundamental y Clinical Pharmacology*, *35*(1), 113-130.

National Research Council (US) Subcommittee on Laboratory Animal Nutrition. (1995). Nutrient Requirements of Laboratory Animals: Fourth Revised Edition. National Academies Press, Estados Unidos.

Pini, R.T.B., Ferreira do Vales, L.D.M., Braga Costa, T.M., Almeida, S.S. (2017) Effects of cafeteria diet and high fat diet intake on anxiety, learning and memory in adult male rats. *Nutr Neurosci* 20 (7):396–408.

Pomrenze, M. B., Fetterly, T. L., Winder, D. G., & Messing, R. O. (2017). The Corticotropin Releasing Factor Receptor 1 in Alcohol Use Disorder: Still a Valid Drug Target?. Alcoholism, clinical and experimental research, 41(12), 1986–1999.

Raubenheimer, D., & Simpson, S. J. (2023). Protein appetite as an integrator in the obesity system: the protein leverage hypothesis. Philosophical Transactions of the Royal Society B, 378(1888), 20220212.

Rojas-Carvajal, M., Leandro, R, & Brenes, J.C. (2022). Distinct acute stressors exert an antagonistic effect on complex grooming during novelty habituation in rats. *Behavioural Processes,* 104931.

Rojas-Carvajal, M., Sequeira-Cordero, A., & Brenes, J. C. (2020). Neurobehavioral effects of restricted and unpredictable environmental enrichment in rats. *Frontiers in pharmacology*, *11*, 674.

Sack, M., Lenz, J.N., Jakovcevski, M., Biedermann, S.V., Falfán, L., Melgoza, C., Deussing, J., Bielohuby, M., Bidlingmaier, M., Pfister, F., Stalla, G.K., Sartorius, A., Gass, P., Weber-Fahr, W., Fuss, J., Auer, M.K. (2017) Early effects of a high-caloric diet and physical exercise on brain volumetry and behavior: a combined MRI and histology study in mice. *Brain Imaging Behav* 11(5):1385–1396.

Sampey, B.P., Vanhoose, A.M., Winfield, H.M., Freemerman, A.J., Muehlbauer, M.J; Fueger, P.T…, Makowski, L. (2011). Cafeteria diet is a robust model of human metabolic syndrome with liver and adipose inflammation: comparison to high-fat diet. *Obesity*, 19, 1109–1117.

Schneider, M. (2013). Adolescence as a vulnerable period to alter rodent behavior. *Cell Tissue Res*, 354, 99–106.

Sclafani, A., Springer, D. (1976). Dietary obesity in adult rats: similarities to hypothalamic and human obesity syndromes. *Physiol Behav*, 17, 461–471.

Sequeira-Cordero A. & Brenes, J.C. (2021). Time-dependent changes in striatal monoamine levels and gene expression following single and repeated amphetamine administration in rats. *Eur J Pharmacol* 904.

Sequeira-Cordero, A., Masís-Calvo, M., Mora-Gallegos, A., Fornaguera, J. (2013). Maternal behavior as an early modulator of neurobehavioral offspring responses by Sprague-Dawley rats. *Behav Brain Res* 237:63–70.

Sequeira-Cordero, A., Salas-Bastos, A., Fornaguera, J., Brenes, J.C. (2019). Behavioural characterisation of chronic unpredictable stress based on ethologically relevant paradigms in rats. *SciRep* 9:1–21.

Shafat, A., Murray, B., Rumsey, D. (2009) Energy density in cafeteria diet induced hyperphagia in the rat. Appetite 52:34–38.

Shizgal, P. (1997) Neural basis of utility estimation. *Current opinion in neurobiology*, 7(2):198-208.

Smith, K. R., & Moran, T. H. (2021). Gastrointestinal peptides in eating-related disorders. *Physiology & Behavior*, *238*, 113456.

Sonko, B. J., Prentice, A. M., Murgatroyd, P. R., Goldberg, G. R., Van de Ven, M. L., & Coward, W. A. (1994). Effect of alcohol on postmeal fat storage. *The American journal of clinical nutrition*, *59*(3), 619-625.

Speliotes, E.K., Willer, C.J., Berndt, S.I., Monda, K.L., Thorleifsson, G., Jackson, A.U…, Loos, R.J. (2010). Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat Genet,* 42, 937–948.

Steele, E. M., Raubenheimer, D., Simpson, S. J., Baraldi, L. G., & Monteiro, C. A. (2018). Ultra-processed foods, protein leverage and energy intake in the USA. *Public health nutrition*, *21*(1), 114-124.

Sturman, O., Germain, P. L., y Bohacek, J. (2018). Exploratory rearing: a context-and stress-sensitive behavior recorded in the open-field test. *Stress*, *21*(5), 443-452.

Sutter, P.M., Hasler, E., Vetter, W. (1997). Effects of alcohol on energy metabolism andbody weight regulation: Is alcohol a risk factor for obesity? *Nutr Rev*, 55, 155–171.

Takase, K., Tsuneoka, Y., Oda, S., Kuroda, M., & Funato, H. (2016). High-fat diet feeding alters olfactory-, social-, and reward-related behaviors of mice independent of obesity. Obesity, 24(4), 886-894.

Teixeira, A. E., Rocha-Gomes, A., Dos Santos, T. P., Amaral, B. L. S., da Silva, A. A., Malagutti, A. R., ... y Riul, T. R. (2020). A cafeteria diet administered from lactation to adulthood promotes a change in risperidone sensitivity on anxiety, locomotion, memory, and social interaction of Wistar rats. *Physiology y behavior*, *220*, 112874.

Tomiyama, A. J., Dallman, M. F., & Epel, E. S. (2011). Comfort food is comforting to those most stressed: evidence of the chronic stress response network in high stress women. *Psychoneuroendocrinology*, *36*(10), 1513-1519.

Tremblay, A., & St-Pierre, S. (1996). The hyperphagic effect of a high-fat diet and alcohol intake persists after control for energy density. *The American journal of clinical nutrition*, *63*(4), 479-482.

Tremblay, J., & Hamet, P. (2019). Environmental and genetic contributions to diabetes. *Metabolism*, *100*: 1-6.

Valladolid-Acebes, I., Fole, A., Martín, M., Morales, L., Victoria Cano, M., Ruiz-Gayo, M., Del Olmo, N. (2013) Spatial memory impairment and changes in hippocampal morphology are triggered by high-fat diets in adolescent mice. Is there a role of leptin?. *Neurobiol Learn Mem* 106:18–25.

Vindas-Smith, R., Quesada, D., Hernández-Solano, M. I., Castro, M., Sequeira-Cordero, A., Fornaguera, J., ... & Brenes, J. C. (2022). Fat Intake and obesity-related parameters predict striatal BDNF gene expression and dopamine metabolite levels in cafeteria diet-fed rats. *Neuroscience*, *491*, 225-239.

Virtuoso, A., Tveden-Nyborg, P., Marie, A., Schou-Pedersen, V., Lykkesfeldt, J., Kaastrup, Muller, H., Elfving, B., Srensen, D.B. (2022) A long-term energy-rich diet increases prefrontal BDNF in Sprague-Dawley rats. *Nutrients* 14:126.

Wang, H., Xu, J., Lazarovici, P., Quirion, R. & Zheng, W. (2018) cAMP Response Element-Binding Protein (CREB): A Possible Signaling Molecule Linkin the Pathophysiology of Schizophrenia. Front. Mol. Neurosci. 11:255.

Wang, Y. J., Liu, L., Wang, Y., Wang, J. L., Gao, T. T., Wang, H. & Jiang, B. (2020). Imipramine exerts antidepressant-like effects in chronic stress models of depression by promoting CRTC1 expression in the mPFC. *Brain Research Bulletin*, *164*, 257-268.

Wise, R.A. (2000). Addiction becomes a brain disease. *Neuron*, 26: 27-33.

World Health Organization. (2014). Global Status Report on Alcohol and Health, 2014. WHO: Geneva, Switzerland. Recuperado de: http://apps.who.int/iris/bitstream/handle/10665/112736/9789240692763_eng.pdf;jsessionid=95CB563DDFB243DD6FCBA3FEA179D681?sequence=1

Yeomans, M. R., Blundell, J. E., & Leshem, M. (2004). Palatability: response to nutritional need or need-free stimulation of appetite?. *British Journal of Nutrition*, *92*(S1), S3-S14.

Yeomans, M.R. (2010). Alcohol, appetite and energy balance: Is alcohol intake a risk factor for obesity? *PhysiolBehav*, 100, 82–89.

Yeomans, M.R., Hails, N.J., Nesic, J.S. (1999). Alcohol and the appetizer effect. *Behav Pharmacol*, 10, 151–161.

CONCLUSIONES GENERALES

- La dieta de cafetería causó un incremento significativo en el consumo de alimento desde la primera semana de exposición a las dietas, en concordancia, el consumo energético de los grupos expuestos a esta dieta fue mayor. Por tanto, como ha sido reportado extensamente en la literatura, la hiperfagia observada se debió principalmente a la alta palatabilidad y variabilidad de la dieta de cafetería que permitió mantener el incremento en el consumo de forma relativamente constante a través del tiempo.

- Los animales expuestos a la dieta de cafetería mostraron un mayor consumo de grasa y carbohidratos, y un menor consumo de proteínas y fibra. El consumo de grasa y azúcar fueron las variables más afectadas por la dieta. Este patrón de ingesta de macronutrientes también contribuye con la hiperfagia, ya que el mismo se ha relacionado con una menor saciedad.

- La dieta de cafetería indujo alteraciones biométricas en los animales en el peso final y en la ganancia de peso corporal. Asimismo, la acumulación de grasa corporal, tanto abdominal como perigonadal fueron las variables biométricas más afectadas por la dieta. La adiposidad visceral se relaciona con las complicaciones metabólicas asociadas a la obesidad, lo que confirma la utilidad del modelo.

- El alcohol no modificó el patrón de ingesta de alimento de los animales, sin embargo, tuvo un efecto en la acumulación de grasa corporal, principalmente en los depósitos de grasa de la región abdominal, impactando a su vez la acumulación de tejido adiposo total. Lo anterior concuerda con la literatura que muestra una relación entre el consumo de alcohol y un aumento de la adiposidad central en humanos, independientemente de la dieta. Sin embargo, el efecto inducido por el alcohol fue menor en comparación con la magnitud del efecto inducido por la dieta de cafetería.

- Los animales expuestos tanto a la dieta de cafetería como al alcohol presentaron un menor consumo de alcohol que los expuestos a la dieta estándar. Esta disminución

podría deberse a un mecanismo compensatorio para regular la ingesta energética y/o a un menor valor reforzante del alcohol frente a la dieta de cafetería.

- A nivel conductual tanto la dieta de cafetería, y en menor medida el alcohol, disminuyeron la actividad exploratoria horizontal de los animales. Los animales expuestos a la dieta de cafetería presentaron una mayor conducta de acicalamiento. De manera que tanto la dieta de cafetería como el alcohol ejercen un efecto de tipo ansiolítico leve en el campo abierto.

- A nivel cerebral, la dieta de la cafetería y el alcohol ejercieron acciones independientes y de interacción sobre la expresión génica y los niveles de proteínas.

- La dieta de cafetería indujo un incremento de los niveles de ARNm de CREB en el HPC y una disminución en la CPFm. Dado que CREB es un regulador crítico en los procesos de plasticidad neuronal, lo anterior evidencia que los cambios inducidos en este proceso son dependientes de la región y aunque opuestos, contribuyen al mismo fenómeno. Por tanto, los niveles elevados de CREB en la CPFm se asocian con la hiperfagia como resultado de alteraciones en el control inhibitorio, mientras que los niveles aumentados en el HPC se relacionan con una mayor reactividad a las señales contextuales que se asocian con la comida, promoviendo la hiperfagia.

- El consumo de alcohol disminuyó los niveles de CREB y TrkB en el HPC, lo que se ha asociado con una disminución de la vía de señalización dependiente de BDNF como un mecanismo protector para contrarrestar los efectos neurotóxicos ejercidos por el alcohol.

- En cuanto al contenido de proteínas, ambos tratamientos redujeron en la misma proporción la proteína CREB en el DS. Sin embargo, el alcohol, y en menor medida la dieta de la cafetería, aumentaron la relación pCREB/CREB en la mPFC y el DS.

- Considerando las diferencias observadas en los niveles de mRNA y proteína, CREB, y en segundo lugar la expresión de CRFR1, fueron los parámetros más sensibles a ambos tratamientos.

- En conjunto, estos hallazgos sugieren que nuestro modelo es útil y confiable para estudiar los efectos neuroconductuales de una dieta occidentalizada moderna sobre el riesgo de sufrir trastornos metabólicos y obesidad. Con este trabajo se concluye además que los objetivos, tanto general como específicos, fueron cumplidos en su totalidad.

REFERENCIAS

Adam, T. C., & Epel, E. S. (2007). Stress, eating and the reward system. *Physiology & behavior*, *91*(4), 449-458.

Barrett, P., Mercer, J.G., Morgan, P.J. (2016). Preclinical models for obesity research. *Dis Model Mech*, 9,1245–1255.

Bellisle, F., Lucas, F., Amrani, R., & Le Magnen, J. (1984). Deprivation, paiatability and the micro-structure of meals in human subjects. *Appetite*, *5*(2), 85-94.

Berthoud, H.R., Lenard, N.R., Shin, A.C. (2001). Food reward, hyperphagia, and obesity. *Am J Physiol Regul Integr Comp Physiol*, 300, 1266–1277.

Blumenthal, D. M., & Gold, M. S. (2010). Neurobiology of food addiction. *Current Opinion in Clinical Nutrition & Metabolic Care*, *13*(4), 359-365.

Bocarsly, M. E., & Avena, N. M. (2013). A high-fat diet or galanin in the PVN decreases phosphorylation of CREB in the nucleus accumbens. *Neuroscience*, *248*, 61-66.

Campos-Rosini, T., Sánchez-Ramos, S.A., de Moraes, C. (2012). Diet-induced obesity: rodent model for the study of obesity-related disorders. *Rev Assoc Med Bras*, 58, 383–387.

Changizi, M.A., McGehee, R.M., Hall, W.G. (2002). Evidence that appetitive responses for dehydration and food-deprivation are learned. Physiology & behavior, 75(3):295-304.

Chao, M.V. (2003). Neurotrophins and their receptors: A convergence point for many signalling pathways. *Nat. Rev. Neurosci*., 4:299–309.

D'Ardenne, K., McClure, S.M., Nystrom, L.E., Cohen, J.D. (2008). BOLD responses reflecting dopaminergic signals in the human ventral tegmental area. Science, 319(5867):1264-1267.

De Castro, J. M. (2000). Eating behavior: lessons from the real world of humans. *Nutrition*, *16*(10), 800-813.

Drewnowski, A. (1998). Energy density, palatability, and satiety: implications for weight control. *Nutrition reviews*, *56*(12), 347-353.

Fernández-Espejo, E. (2002). Bases neurobiológicas de la drogadicción. *Revista de neurología, 34 (7), 659-664*.

Fisberg, M., Kovalskys, I., Gomez, G., Rigotti, A., Cortés, L. Y., Herrera-Cuenca, M., ... & Chiavegatto Filho, A. D. P. (2015). Latin American Study of Nutrition and Health (ELANS): rationale and study design. *BMC Public Health*, *16*(1), 93.

German, D.C., Manaye, K.F. (1993). Midbrain dopaminergic neurons (nuclei A8, A9, and A10): three-dimensional reconstruction in the rat. *J CompNeurol*, 331, 297–309.

Gutiérrez-Martos, M., Girard, B., Mendonça-Netto, S., Perroy, J., Valjent, E., Maldonado, R., Martin, M. (2018). Cafeteria diet induces neuroplastic modifications in the nucleus accumbens mediated by microglia activation. *Addict Biol,* 23, 735–749.

Huang, E.J., Reichardt, L.F. (2001). Neurotrophins: roles in neuronal development and function. *Annual Review of Neuroscience*, 24: 677–736.

Koob, G. F., & Volkow, N. D. (2016). Neurobiology of addiction: a neurocircuitry analysis. *The Lancet Psychiatry*, *3*(8), 760-773.

Kovalskys, I. Fisberg, M., Gómez, G. Pareja, R. Yépez García, M., Cortés Sanabria, L., Koletzko, B. (2018). Energy intake and food sources of eight Latin American countries: results from the Latin American Study of Nutrition and Health (ELANS). *Public Health Nutrition*, 21(14), 2535-2547.

Kyzar, E. J., & Pandey, S. C. (2015). Molecular mechanisms of synaptic remodeling in alcoholism. *Neuroscience letters*, *601*, 11-19.

Lu, B., Nagappan, G., Guan, X., Nathan, P.J., Wren, P. (2013). BDNF-based synaptic repair as a disease-modifying strategy for neurodegenerative diseases. *Nat. Rev. Neurosci*. 2013; 14:401–416.

Lyon, H.N., Hirschhorn, J.N. (2005). Genetics of common forms of obesity: a brief overview. *Am J Clin Nutr*, 82, 215S–217S.

Ministerio de Salud y Ministerio de Educación Pública. (2017). Informe Ejecutivo Censo Escolar Peso/Talla. Costa Rica 2016. Recuperado de http://www.mep.go.cr/sites/default/files/page/adjuntos/informe-ejecutivo-censo-escolar-peso-cortofinal.pdf

Morris, M.J., Beilharz, J.E., Maniam, J., Reichelt, A.C., Westbrook, R.F. (2015). Why is obesity such a problem in the 21st century? The intersection of palatable food, cues and reward pathways, stress, and cognition. *Neurosci Bio behav Rev*, 58, 36–45.

Ohira, K., Hayashi, M. (2009). A new aspect of the TrkB signaling pathway in neural plasticity. *Curr. Neuropharmacol*., 7:276–285.

Olsen, C.M. (2011). Natural rewards, neuroplasticity, and non-drug addictions. *Neuropharmacology*, 61, 1109–1122.

Pandey, G. N., Ren, X., Rizavi, H. S., Conley, R. R., Roberts, R. C., & Dwivedi, Y. (2008). Brain-derived neurotrophic factor and tyrosine kinase B receptor signalling in post-mortem brain of teenage suicide victims. *The The International Journal of Neuropsychopharmacology*, *11*(8), 1047-1061.

Phillips, A.G., Vacca, G., Ahn, S. (2008). A top-down perspective on dopamine, motivation and memory. *Pharmacology, biochemistry, and behavior*, 90(2):236-249.

Popkin, B.M., Reardon, T. (2018). Obesity and the food system transformation in Latin America. *Obesity*, 19, 1028–1064.

Roca, A. J. (2010). Estrés y enfermedad. Enfoque psiconeuroinmunoendocrino. *Medicina*, *32*(3), 223-236.

Rogers, P. J. (1990). Why a palatability construct is needed. *Appetite*, *14*(3), 167-170.

Sampey, B.P., Vanhoose, A.M., Winfield, H.M., Freemerman, A.J., Muehlbauer, M.J; Fueger, P.T…, Makowski, L. (2011). Cafeteria diet is a robust model of human metabolic syndrome with liver and adipose inflammation: comparison to high-fat diet. *Obesity*, 19, 1109–1117.

Schultz, W. (2010). Subjective neuronal coding of reward: temporal value discounting and risk. *European Journal of Neuroscience*, *31*(12), 2124-2135.

Sclafani, A., Springer, D. (1976). Dietary obesity in adult rats: similarities to hypothalamic and human obesity syndromes. *Physiol Behav*, 17, 461–471.

Shizgal, P. (1997) Neural basis of utility estimation. *Current opinion in neurobiology*, 7(2):198-208.

Smith, Y., Villalba, R. (2008). Striatal and extrastriatal dopamine in the basal ganglia: an overview of its anatomical organization in normal and Parkinsonian brains. *Mov Disord*, 23(Suppl 3): S534-547.

Speliotes, E.K., Willer, C.J., Berndt, S.I., Monda, K.L., Thorleifsson, G., Jackson, A.U, Loos, R.J. (2010). Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat Genet,* 42, 937–948.

Sutter, P.M., Hasler, E., Vetter, W. (1997). Effects of alcohol on energy metabolism andbody weight regulation: Is alcohol a risk factor for obesity? *Nutr Rev*, 55, 155–171.

Taché, Y., Martinez, V., Wang, L., & Million, M. (2004). CRF1 receptor signaling pathways are involved in stress-related alterations of colonic function and viscerosensitivity: implications for irritable bowel syndrome. *British journal of pharmacology*, *141*(8), 1321-1330.

Vindas-Smith, R., Quesada, D., Hernández-Solano, M. I., Castro, M., Sequeira-Cordero, A., Fornaguera, J., ... & Brenes, J. C. (2022). Fat Intake and obesity-related parameters predict striatal BDNF gene expression and dopamine metabolite levels in cafeteria diet-fed rats. *Neuroscience*, *491*, 225-239.

Wang, H., Xu, J., Lazarovici, P., Quirion, R., & Zheng, W. (2018). cAMP response element-binding protein (CREB): a possible signaling molecule link in the pathophysiology of schizophrenia. *Frontiers in molecular neuroscience*, *11*, 255.

Wise, R.A. (2000). Addiction becomes a brain disease. *Neuron*, 26: 27-33.

Wise, R.A. (2005). Forebrain substrates of reward and motivation. *The Journal of comparative neurology*, 493(1):115-121.

World Health Organization. (2014). Global Status Report on Alcohol and Health, 2014. WHO: Geneva, Switzerland. Recuperado de: http://apps.who.int/iris/bitstream/handle/10665/112736/9789240692763_eng.pdf;jsessionid=95CB563DDFB243DD6FCBA3FEA179D681?sequence=1

Yeomans, M. R., Blundell, J. E., & Leshem, M. (2004). Palatability: response to nutritional need or need-free stimulation of appetite?. *British Journal of Nutrition*, *92*(S1), S3-S14.

Yeomans, M.R. (2010). Alcohol, appetite and energy balance: Is alcohol intake a risk factor for obesity? *PhysiolBehav*, 100, 82–89.

Yeomans, M.R., Hails, N.J., Nesic, J.S. (1999). Alcohol and the appetizer effect. *Behav Pharmacol*, 10, 151–161.